JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY

JAOCS

Volume 57 / Number 9 / September 1980

D

Oilseed studies
at Russell Research Center
focus on sunflower


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Calendar

WORLD CONFERENCE

Nov. 9-14, 1980 – World Conference on Soya Processing and Utilization, Acapulco Convention Center, Acapulco, Mexico.

AOCS NATIONAL MEETINGS

Annual Meeting, 1981: May 17-21, Fairmont Hotel, New Orleans, LA.

Annual Meeting, 1982; May 2-6, Sheraton Centre, Toronto, Ontario, Canada.

Annual Meeting, 1983: May 8-12, Chicago Marriott, Chicago, IL.

1980

September

Chemical Marketing Research Association, Sept. 21-24, 1980, Doral, Country Club, Miami, Florida, Contact: CMRA, 139 Chestnut Ave., Staten Island, New York, 10305.

"NACE Basic Corrosion Course," sponsored by NACE, Sept. 21-26, 1980, University of Alabama, Tuscaloosa, AL. Contact: Education Dept., NACE Headquarters, PO Box 218340, Houston, TX 77218.

11th International Federation of Societies of Cosmetic Chemists Congress, Sept. 23-26, 1980, Venice Lido, Italy. Contact: M. Fadini, Organizing Secretariat, 11th International I.F.S.C.C. Congress, c/o Studio MGR, Piazza Sant 'Ambrogio 16, 20123 Milan, Italy.

1980 Conference on International Cosmetic Regulations, Venice, Italy, Sept. 27, 1980, sponsored by the International Federation of Societies of Cosmetic Chemists.

ASTM Committee E-13 on Molecular Spectroscopy, sponsored by ASTM, Sept. 28-30, 1980, ASTM Headquarters, Philadelphia, PA. Contact: J.R. Schroeder, ASTM, 1916 Race St., Philadelphia, PA 19103.

"Hydrogenation of Oils," sponsored by the Italian Society for Fats Research, Sept. 29-30, 1980, Italy. Contact: Secretary of the Symposium, c/o Prof. Enzo Fedeli, Stazione Sperimentale Oli e Grassi, Via Giuseppe Colombo, 79-20133, Milan, Italy.

Seventh Annual Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies, Sept. 28-Oct. 3, 1980, Philadelphia Sheraton, Philadelphia, PA. Contact:

Continued on page 702A.

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Russell Center focuses on sunflower oilseed research

Perched atop the tallest of a cluster of gently rolling hills on the outskirts of Athens, Georgia, the USDA's Richard B. Russell Research Center forms part of the University of Georgia Research Park.

With the Southern Regional Research Center in New Orleans, the Russell Center shares responsibility for utilization research on agricultural production in the South—an area that extends roughly from the Atlantic coast westward to Texas and from the Ohio River south to the Gulf Coast. While the geographic jurisdiction is shared, the responsibility for oilseed research is not. New Orleans researchers concentrate on cottonseed and peanuts, two major oilseed crops of the South. Athens researchers focus on sunflower and have an additional project on problems unique to on-farm storage of soybeans in the Southeast. (Most USDA post-harvest utilization research on soybeans is conducted at the Northern Regional Research Center in Peoria, Illinois).

Dr. James Robertson, oilseed research leader at the Russell Center, explains that when the center was opened in 1969, sunflower was viewed as a potential diversification crop. The late Dalton Gandy, then with National Cottonseed Products Association, was spearheading the attempt to introduce sunflower to the South. Thus, one of the Russell Center's original tasks was sunflower research and Robertson was a chartered staff member. For two years, he was the sole oilseed researcher. Dr. W.H. Morrison III joined the staff in 1971, and the two men continue as the Center's principal sunflower investigators.

Robertson began by trying to determine the characteristics of sunflower oil grown in the United States. An extensive network of cooperators was established to keep data on planting dates, locations and weather conditions, which Robertson would correlate with sunflower yield and sunflower oil composition. The results showed that growers can "pretty well tailor grow an oil," Robertson says, as southern grown sun oil's fatty acid profile (35–50% linoleic acid) showed it suitable as a frying oil for the snack and food industry whereas northern grown oil (65–70% linoleic acid) is suitable for salad oil and similar uses. Temperature and planting location are significantly correlated with sun

Robertson: "On-farm storage facilities have increased 300% since 1977, and with the accumulation of U.S. soybean stocks, it is essential that adequate information be developed on both short- and long-term storage."

oil's fatty acid composition. In general, for each centigrade-degree increase in average minimum daily temperature from flowering of the sunflower until maturity, linoleic acid content of the seed decreases by about 2%. The results have been published in a USDA bulletin, *Effect of Planting Location and Temperature on the Oil Content and Fatty Acid Composition of Sunflower Seeds* (USDA, SEA, Agricultural Research Results ARR-S-3/ October 1979).

Some of the Russell Center's early work involved examining the suitability of sun oil for frying potato chips or preparing frozen french fries. The reason is that potatoes and sunflowers are grown in the same general region of the U.S. and provide cooperatives and others the possibility of using readily available raw materials. The work on french fries showed sun oil, despite a low

initial active oxygen method (AOM) value, has about the same AOM value after equilibrium status was reached as soy-based and palm-based drying oils. Robertson has copies of publications and a presentation of a 1978 international sunflower conference on these topics.

Rapidly increasing sunflower production and processing in the U.S. will lead to increasing on-farm storage of seed. "We do not have adequate information on optimum conditions for (on-farm) storage of sunflower," Robertson notes. Grain buyers are asking how much damage can be expected under various storage conditions, at what free fatty acid level they should reject sunflower seed and similar questions. An initial analysis from 54 storage bins on 27 farms showed that there were significant storage problems with seed that was placed in storage at high moisture contents (above 10%) and improperly aerated.

Storage conditions have been the subject of the soybean research at the center, specifically, what physiological effects on soybeans are caused by storage at the high-temperature, high-humidity conditions common in the Southeast. The Russell work showed that the normal practice of storage of 13-13.5% moisture may not be best in the Southeast; moisture probably should be 12% or below. "On-farm storage facilities have increased 300% since 1977, and with the accumulation of U.S. soybean stocks, it is essential that adequate information be developed on both short- and long-term storage," Robertson says. The soybean work also involved developing a new analytical phospholipid conversion factor by determining individual phospholipid molecular weight based on fatty acid composition. The work also applied to sunflower, the first time such a factor had been developed for crude sunflower oils.

Recent cutbacks in federal ag utilization research funds have forced changes at all the regional research centers, usually involving curtailment of staff and research projects.

In fact, the Russell team has had to develop several analytical methods for sunflower. There are now three tentative methods, adopted in 1975, for sunflower (moisture and volatile matter, oil, and nitrogen-ammonia-protein) in *AOCS Official and Tentative Methods.* But as Robertson says, "there are no industry-wide accepted methods and different methods are being used."

The team is looking for faster, better methods for oil analysis. Wideline nuclear magnetic resonance (NMR), for example, can provide more rapid analysis than the extraction techniques the AOCS tentative method uses, with equivalent results. More recent work has explored use of near infrared (NIR) analysis, but with less favorable initial results. In working with NMR, Robertson says his work shows drying samples at 130 C for three hours is unnecessary, that 30 minutes may be enough; samples exposed to a microwave for four minutes or longer also was tried, but results were spotty.

Robertson serves as chairman of the AOCS sunflower seed analysis committee. With increasing commercial trading of sunflower, development of industry-wide accepted analytical methods becomes important.

One analytical project seeks to identify and characterize sunflower seed waxes and their effects on oil quality. The goal is to investigate the composition and location of waxes and other minor constituents and what can be done about them. In small amounts, the waxes are an aesthetic problem in that they cause oil to become hazy. The first step is to determine the amount of wax in sunflower hulls, kernels and seed coats. Morrison presented a paper on initial work of this three-year project during the ISF/AOCS World Congress this past spring.

Morrison presented another paper on a second active project: correlating gas liquid chromatography (GLC) volatile measurements with evaluations by taste panels (trained and untrained panel tastings are conducted under supervision of a Russell Center home economist). The project involves testing of fresh oil as well as oils stored for varying amounts of time under different conditions. The work has shown that storage in dark or amber bottles provides adequate flavor protection without the addition of antioxidants. But since the firms presently marketing sun oil consumer products prefer to emphasize the "lightness" of sun oil, they probably will continue to use clear containers, Robertson says. Evaluations of sun oils stored for 16 weeks in clear containers did not show significant flavor protection by antioxidants for sun oil, Robertson notes.

What about sunflower meal? Although somewhat deficient in lysine, sunflower meal contains about twice as much methionine as soybean meal and is

potentially an excellent source of protein for livestock. Research conducted at the Russell Center by Drs. Henry Amos and Donald Burdick showed that a higher percentage of sunflower protein was retained by sheep when the meal was heated with formaldehyde (1%) to "protect" the protein from degradation in the rumen. Also, data obtained indicated that sunflower meal heated to 100 C during processing resulted in faster weight gain in rats than untreated meal or meal heated to 127 C. Two factors which presently preclude greater use of sunflower protein in human foods are the presence of polyphenolic acids and the high fiber content of sunflower meal. Polyphenolic acids, particularly chlorogenic acid, impart a greenish color to foods which is esthetically undesirable. Research funded by the Russell Center at Texas A&M University has been partially successful in solving both problems.

The Russell Center is still relatively new, which has benefits and drawbacks. The facility includes modern design, but it also lacks the capital improvements that build up over time (such as pilot plant facilities). Recent cutbacks in federal ag utilization research funds have forced changes at all the regional research centers, usually involving curtailment of staff and research projects. Reduced travel funds may mean less opportunity to meet with other researchers to discuss, for example, the effect of fertilization on sunflower seed yield and composition.

As sunflower becomes economically more important, Robertson says it could be the only oilseed crop the Russell Center will have time and funds to study. The dilemma, as at every research organization, is that there are many challenging tasks and not enough resources to undertake them all, especially in an era of rising costs and limited funding.□

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Profile: John Monick

Four decades ago, John Monick decided that a career in chemistry "would provide an interesting future." His 42 years helping Colgate Palmolive pioneer developments in the soap and detergent industry have validated his judgment.

"It (chemistry) is creative; you're always producing something new; and you can take pride in your work," he says. "I've always wanted to be doing something, not just sitting behind a desk. I want to be there to see a new plant go up, or see a new process go into production."

His academic and professional career is almost a prototype American success story.

He was born in 1917 on New York's lower East Side, about four years after his parents came to the United States. His father originally was from Russia; his mother was from Poland. "I had an all-around education in that teeming East Side, mixing with kids of all nationalities, that I don't think you could get anywhere else in the world," John says. Junior High School 64 and the local Boys' Club were both close to Tompkins Square park, providing ample opportunity for games, sports and socializing.

When he enrolled at Peter Stuyvesant High School, John already knew he wanted to take as many chemistry courses as he could. Although he was adept at physics and math also, experimenting with a chemistry kit while in junior high school had opened the world of chemistry to him. John did well in all his courses, and his grades earned one of the few available state scholarships, so he enrolled at the City College of New York as a chemical engineering major. He was graduated in 1938, No. 1 in his class at the age of 20.

But 1938 was a Depression year even for top scholars; jobs were scarce. A friend of his father knew of an opening for an analytical chemist at Colgate's facilities in New Jersey just across the Hudson River from New York City. Monick applied and was hired to analyze natural fats and oils and soaps in the firm's research department. He has been with Colgate ever since.

Several important events occurred to Monick during 1941. First, he transferred to Colgate's chemical engineering department, a step which opened up his future career. Second, World War II started and he was assigned to war-related research and thus did not enter military service. Third, at a dance on the lower East Side, where he was still living, John met a vivacious young lady named Lydia Chozianin. They both liked to dance; they had some mutual friends. The two were wed in 1944. Lydia is as outgoing as John is quiet.

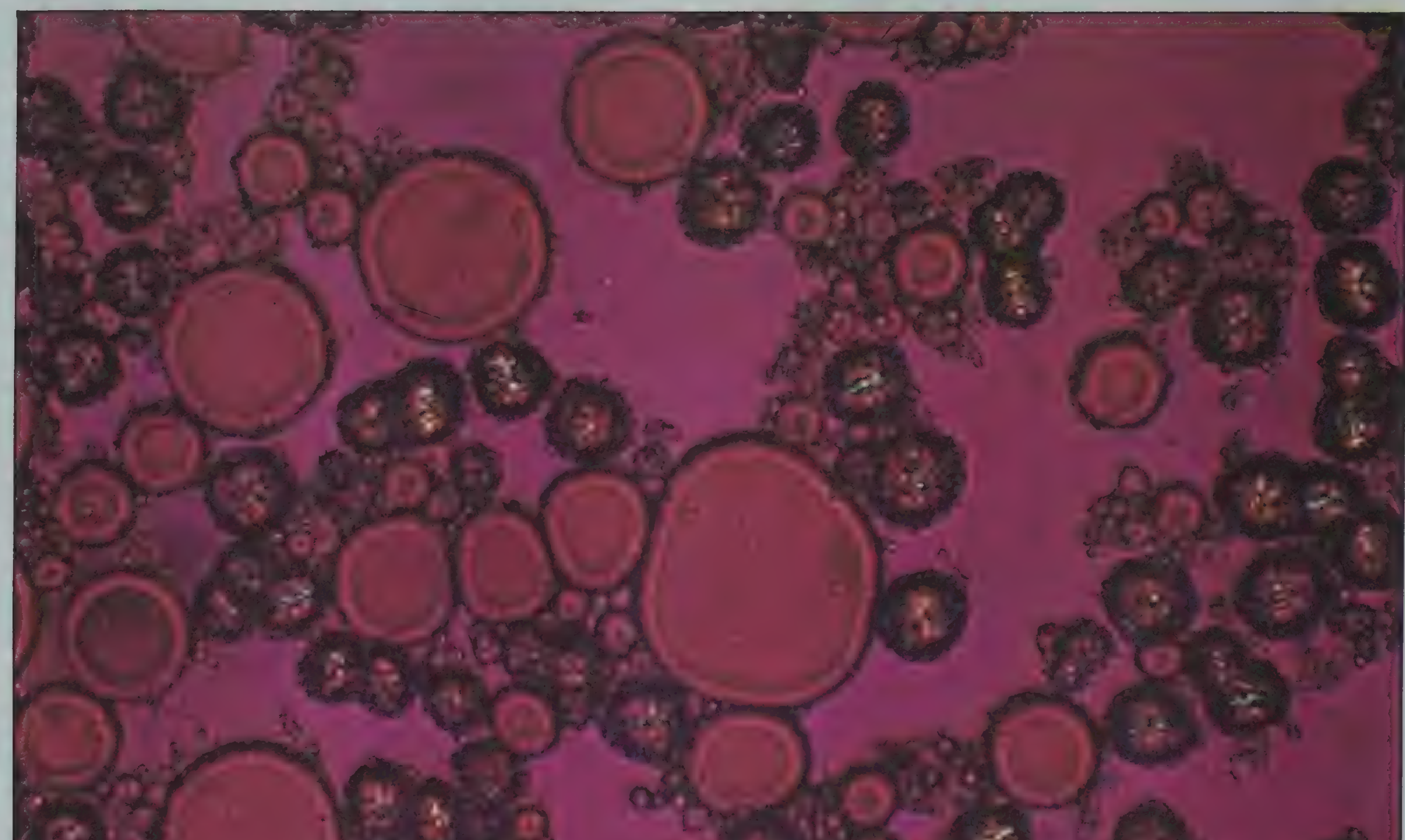
"I guess I get engrossed in my science projects and concentrate on them," John says. "Lydia is very outspoken. In many ways, I have often wished I could be more like her."

Their first daughter, Dianne, was born while they were still living in New York. Now the mother of two children, Dianne lives in New Jersey near Philadelphia. John says she's relatively quiet. Their second daughter, Susan, was born in New Jersey and now is the main spark for a four-member female Blue Grass music group based in Nashville, Tennessee. She is more like Lydia, John says.

At the time they were first married, however, all that was far in the future. John was working at Colgate with other engineers to develop spray drying of natural soap and a practical method to produce laundry products in million-pound quantities. The result was Super Suds, a popular laundry soap after World War II. He also worked for a while with Martin H. Ittner in the original studies at Colgate on countercurrent, continuous high-press hydroly-

Continued on page 680A.

This is the way w
yield of o



Feed to separator.

e improve your
ein by 33%

The conventional methods of separating a natural oil or fat into triglycerides with different degrees of unsaturation are dry fractionation or solvent fractionation.

By these methods, the high-melting stearin is removed from the oil by filtration.

The Lipofrac process

In the Alfa-Laval Lipofrac process, the filtration stage is replaced by centrifugal separation preceded by treatment with water containing a wetting agent.

The basic principle is simple. A partly crystallized fat is mixed with water containing a wetting agent. The surfactant serves to wet the fat crystals which migrate to the aqueous phase, and they are washed free of adherent oil. The picture shows the separator feed. The water suspension of fat crystals is then separated from the lighter oil (olein) phase by centrifugation. This procedure avoids the filtration stage of the conventional methods.

The wetting agent and centrifugal separation make it possible to separate crystals of much smaller size than in other methods, which greatly simplifies the crystallization stage. The necessary crystallization time is only 2–6 hours, depending on the fat or oil.

After the first separation in which the olein is separated out, the fat crystals in the aqueous phase are subsequently melted and the phase is again separated by centrifugation. The light phase consists of stearin, whereas the heavy phase is the aqueous solution, which is recycled to the process.

Typical fats and oils which can be fractionated in Lipofrac are:

- tallow
- lard
- palm oil
- partially hydrogenated soybean oil
- fatty acids.

Lipofrac–no filter, no solvent

The major advantages of the Lipofrac process, as compared to conventional fractionation methods, are:

- crude as well as refined oils can be processed
- the olein yield is high (compared to dry fractionation)
- eliminates filter
- no risk of explosion
- no costs involved in losses and recovery of solvent
- lower investment costs (compared to solvent fractionation)
- lower manpower requirements
- compact design
- easy to operate.

As an alternative, the basic equipment can also be used for alkali refining.

Olein yield: 33% increase

It is assumed that the normal yield of olein from palm oil in Lipofrac is 80%, and that in a dry process it is 60%.

Let us look at an example:

Price of crude palm oil:	a U.S.$/ton	(March 1980)
Price of stearin:	a U.S.$/ton	
Price of olein:	1.2×a U.S.$/ton	

One ton of palm oil will thus give:

$0.80\times1.2\ a+0.20\times a=1.16\ a$ (Lipofrac)
$0.60\times1.2\ a+0.40\times a=1.12\ a$ (dry fractionation)

In the case of crude palm oil, "a" is approx. 650 U.S.$/ton (March 1980) and the difference between the two methods is then $0.04\times650 = 26$ U.S.$/ton.

The normal annual production (300 working days) in a 100 t/24 h is 30,000 ton. The increased profit due to the extra olein yield will thus be:

$$26\times30{,}000 = 780{,}000 \text{ U.S.\$/year}$$

Even if the price of stearin can be expected to increase somewhat as compared to the price of olein, it is still the olein yield which predominantly contributes to the profitability of the plant.

As you can see, this is an easy way to improve the profit of your next fractionation plant. Contact your national Alfa-Laval representative or write direct to the address below.

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sis of fats and oils. "We were somewhat the pioneer of the monoglyceride business in the United States," John says. He designed, supervised the installation of and was process chemist for a Colgate monoglyceride pilot plant in Jersey City that could produce up to one million pounds of product per year for the baking and lubricant industries. Demand from the baking industry quickly reached 10 million pounds per year and Colgate decided not to diversify in that direction.

The spray drying work on natural soaps proved even more successful after World War II, with petroleum-based detergents. Monick did development work on control of particle size and weight that was used to produce Colgate's first spray-dried detergent, Vel.

He also helped develop Gardol, a prefluoride-era toothpaste that used sodium-*n*-laurolysarcosinate as its anticaries agent.

During this time, John had continued his evening studies while working full-time and raising a family. He had received his masters in 1945 from Brooklyn Poly Tech. Now that the family had moved to New Jersey, he was commuting evenings to New York City to pursue a doctorate at New York University, which he received in 1955. "That was 18 years from bachelor's degree to doctorate," John says. "I was the oldest graduate in my class." His parents, especially his mother, always had stressed the importance of education.

With Colgate's "Associated Products" group, Monick helped maintain and improve the quality of existing products as well as develop new ones. Individually packaged, moist paper towels were one development, as was Colgate's line of individual-sized personal care products for hotels. Institutional and industrial products also came from the group.

His definitive book, *Alcohols*, was published in 1968 and remains one of the standard volumes on the subject. He wrote articles on alcohols, phenols and ethers for the third edition of *Encyclopedia Britannica*. He holds more than a dozen patents in the detergent and chemical fields. He has numerous scientific publications. His first publication in an AOCS journal was in *Oil and Soap* during 1946. That journal and AOCS methods first called the society to his attention. He became a member in 1949 and has been active in the Northeast Section since its founding. John was among the half-dozen or so persons who developed the ISF/AOCS World Congress sessions on soaps and detergents.

That took a major effort, for about 2 years ago John was told he has cancer. Colgate named him a senior research associate to relieve him of administrative duties and permit him to continue scientific work. "It's somewhat like a consulting position," he explains. "It gives you the freedom of selecting projects, making suggestions for research programs during the following year, but without the paperwork of being a section head."

He felt well enough to deliver a paper, "The Chemistry of Alcohols," at the American Chemist Society's meeting in Houston earlier this year, but was not strong enough to attend the ISF/AOCS meeting. AOCS friends and associates have praised his courage and determination in facing the disease; Monick says his attitude toward his illness is primarily a result of his "stubborness."

Looking back on his career in chemistry, Monick says if he were a young man facing a career decision today, he'd still choose chemistry. As he says, "Important things are happening in chemistry. Once I had become interested in chemistry, I wasn't interested in anything else." □

Mounts named to lead oilseed research

Timothy L. Mounts has been named Acting Chief, Oilseeds Crops Laboratory at the USDA's Northern Regional Research Center in Peoria, Illinois. The Oilseeds Crops Laboratory is known as the world's leader in soybean research, conducting diverse studies of soybean oil and edible soy protein.

Mounts

Mounts succeeds Herb Dutton, who is now able to devote full time to lab work. Dr. Dutton recently has begun directing a study of photosynthesis in soybeans.

Mounts has been employed at the Peoria lab for 23 years, most recently as Leader, Edible Oils Products and Processes Research Unit. He was technical program chairman of the AOCS short course "Processing and Quality Control of Fats and Oils" in 1978 at East Lansing, Michigan, and in 1980 at Lake Kiamesha, New York. He is a member of the AOCS Flavor Nomenclature Committee and Education Committee and a member of the Board of Governors, North Central Section, AOCS. Currently, Mr. Mounts is serving as a member of both the steering committee and program committee for the 1982 World Conference on Edible Oil Processing. He has over 50 publications in the field of fats and oils.

Tim lives in Peoria with his wife, Eileen, and three children, Peggy, Tim, Jr., and Julie, where he has been active in civic and church affairs and will serve as 1981 chairman, Peoria Section, ACS. □

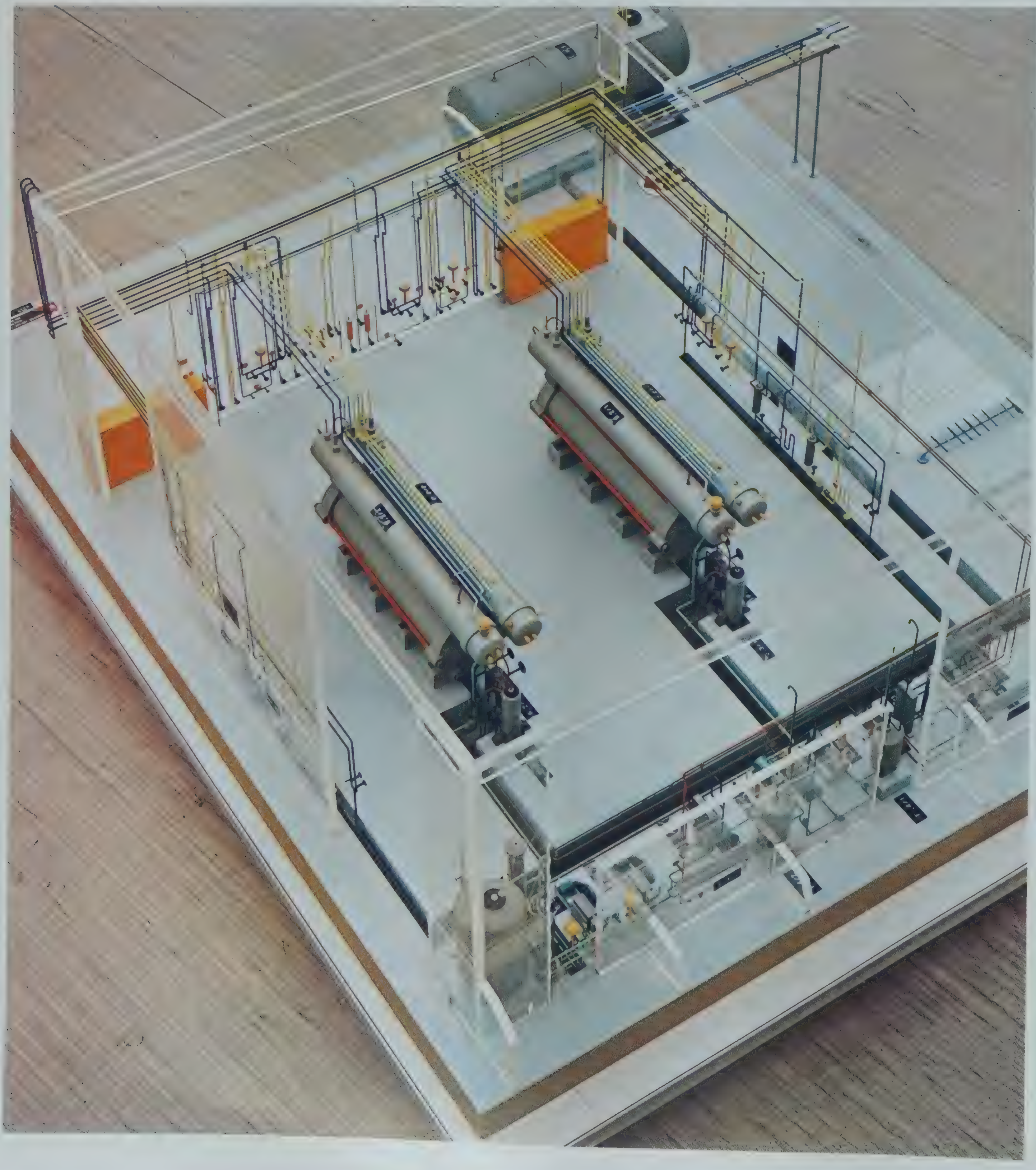
Continued on page 681A.

LURGI

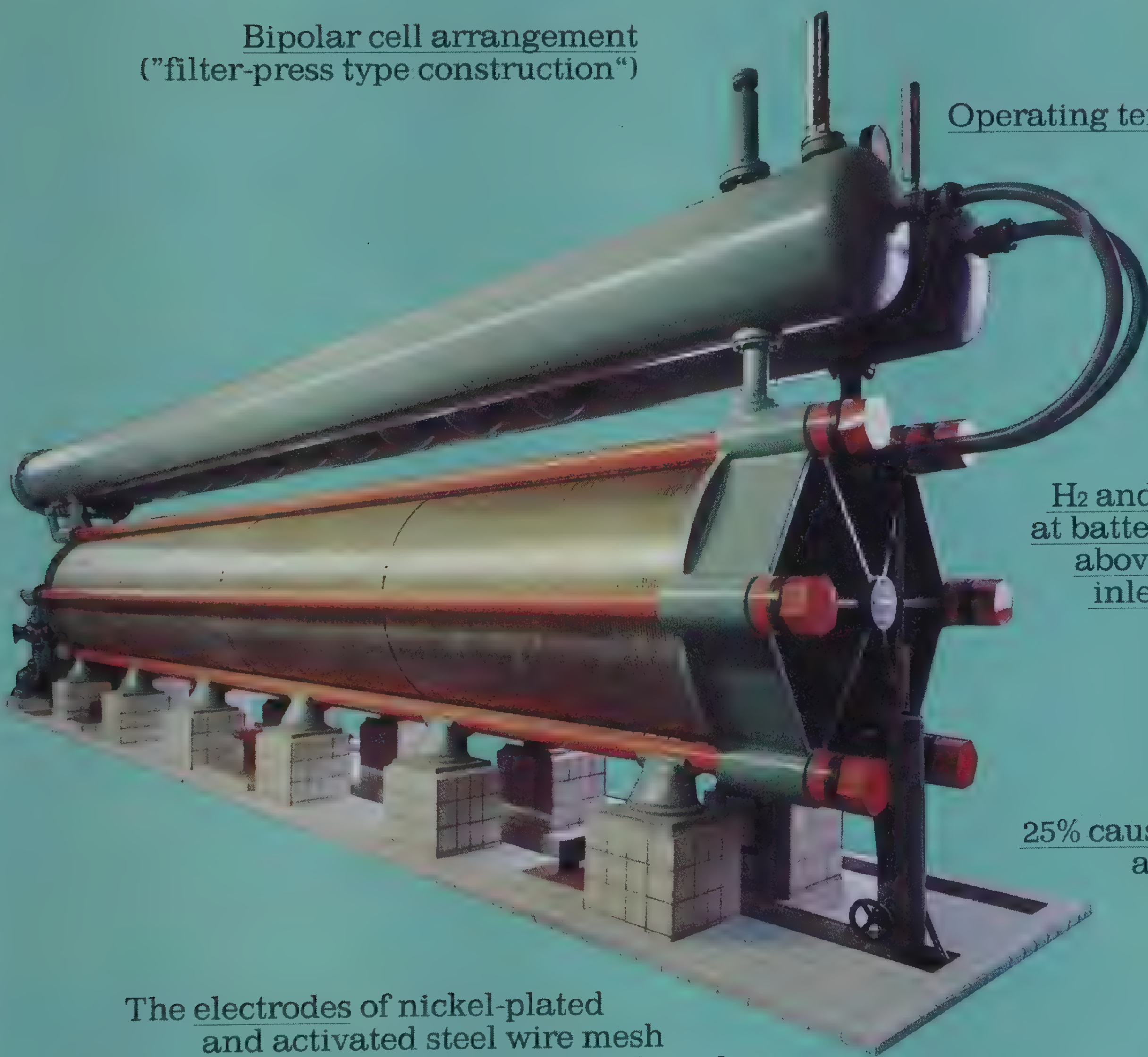
Topic of the day: Production of Hydrogen by Pressure Electrolysis

Hydrogen ex Lurgi pressure electrolysis plant

Model of pressure electrolysis plant



- Low electrical energy consumption: 4.3 – 4.6 kWh per 1 m^3 hydrogen and 0.5 m^3 oxygen at 30 bar (g)
- Storage of gas at pressure up to 30 bar (g) without compression
- Capacity per unit max 760 m^3/h hydrogen, hence low space requirements
- Automation of plants. Even computer control from a distant centralized control room of a factory has proved successful.
- Low cost of personnel through automation
- Flexible capacity variation between 25 and 100% within a matter of seconds, e.g. as a function of the storage pressure
- 40 plants with capacities of 100 to 4,700 m^3/h, some of them operating for more than 20 years, testify to reliability, safety and economics



Operating data ■ Electrical energy consumption of pressure electrolyzer . . .
4.3 – 4.6 kWh pro m³ H_2 + 0.5 m³ O_2 (m³ referred to 0 °C and 1.013 bar)

The energy consumption applies to the gases (H_2 + O_2) compressed to 30 bar (g)

Note: For the compression of 1 m³ H_2 from 0 to 30 bar (g), making allowance for compressor and motor efficiencies, the requirement is . . approx. 0.2 kWh/m³ H_2

- available H_2- and O_2-pressure — 30 bar (g)

- gas purity downstream of pressure electrolysis at nominal capacity: — H_2 . . . 99.8 – 99.9 Vol.%; O_2 . . . 99.3 – 99.6 Vol.%

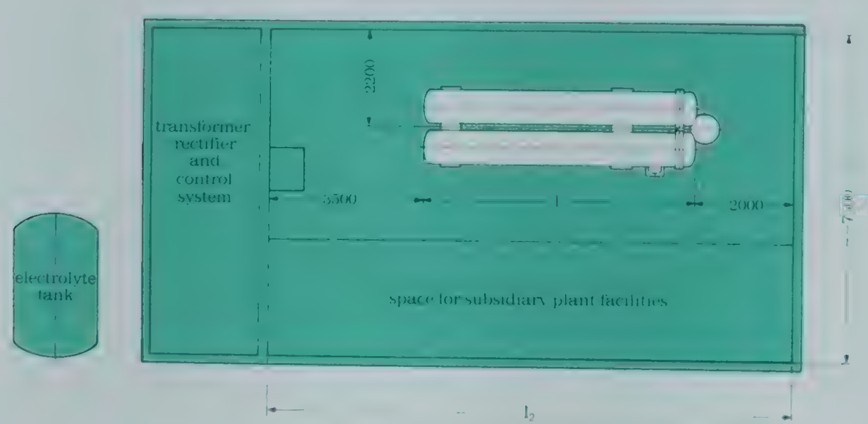
  Contaminants:

O_2 in H_2	0.1 – 0.2 Vol.%
H_2 in O_2	0.4 to 0.7 Vol.%
H_2O as steam and droplets	approx. 1 – 2 g/m³
KOH at limit of detectability	< 0.1 mg/m³
N_2 (dissolved in feed water charged)	approx. 20 vol. ppm

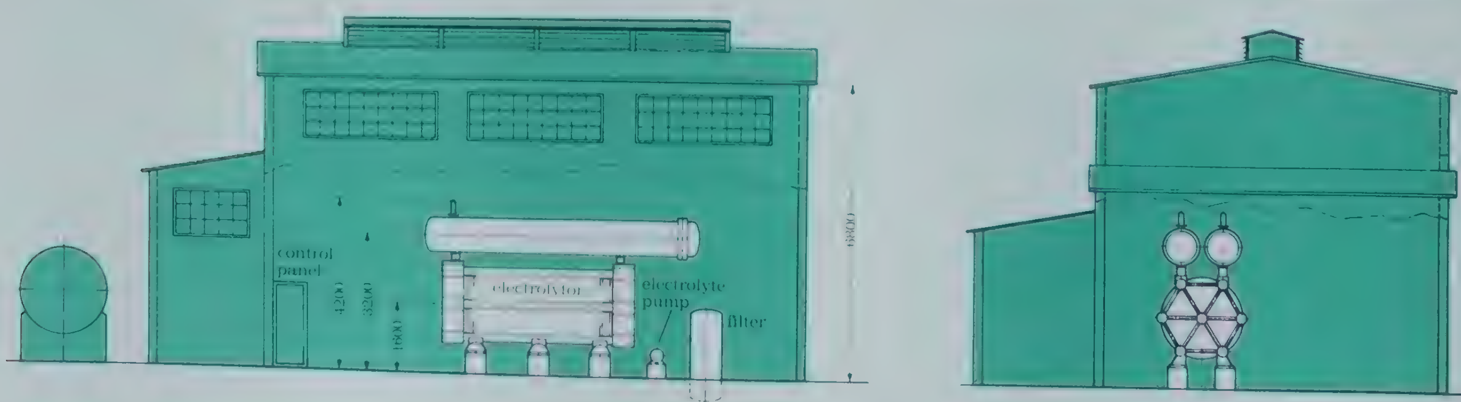
- Gas purity after deoxo and adsorption drying plant, in case feedwater has been degassed —
  H_2 . . . 99.999 Vol.%
  O_2 . . . 99.999 Vol.%
  O_2 in H_2 ≙ attainable < 1 Vol. ppm
  H_2O = attainable < 1 Vol. ppm
  N_2 10 Vol. ppm

- Cooling water consumption (at $\Delta t = 20$ °C) — < 0.04 m³ per m³H_2 + 0.5 m³O_2

Space Requirements of a Pressure Electrolysis Plant



hydrogen Production rate m³H_2/h	number of cells	l_1 mm	l_2 mm
110	81	4300	9800
200	148	5500	11000
300	222	6400	11900
400	296	8100	13600
500	372	9500	15000
600	444	10700	16200
760	556	12500	18000



80 - 4

LURGI

...the plants are built by Lurgi

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Awards presented at Durkee

Durkee Foods, Division of SCM Corporation, recently recognized six employees for outstanding work done in the area of technical and scientific achievement and seven employees who were awarded patents. In addition, the company has announced recent staff changes and appointments.

Those receiving scientific and technical achievement awards were Mark H. Otterson, Steven M. Rikon, Douglas W. Perkins, Robert R. Delaney, Himus S. Sims and Juris A. Kelers. Those who received awards for patents were A.V. Petricca, A.S. Hodgson, Edmund H. Becker, Robert O. Lindstrom, C.E. Rule, D.E. Miller and E.E. Werstak.

Appointments included Christine L. Ford and Donald E. Kindstrand as technical sales representatives for the Midwest region and Wendy S. Thompson and J. Scott Benincasa as territorial sales representatives for the Midwest region.

Also in the Midwest region, Robert O. Benson was named a bulk sales field representatitive. Denis F. Mochan was appointed to the position of market manager—Europe and Charles E. Rule has become manager—national technical service. In addition, Millard M. Evak has been appointed vice-president of trading and Kim W. Kuback has been appointed manager of national accounts and technical service, Eastern region. □

IFT confers honors

Dr. F. Jack Francis, professor of food science at the University of Massachusetts, was named president of the Institute of Food Technologists, a 20,000-member scientific society, at the organization's 40th annual meeting held in June in New Orleans. Dr. John C. Ayres, professor of food science at the University of Georgia, was presented the 1980 International Award at the IFT meeting. □

Pomeranz receives Wiley Award

Dr. Yeshajahu Pomeranz, director of the U.S. Grain Marketing Research Center in Manhattan, KS, has been selected to receive the 1980 Wiley Award by the Association of Official Analytical Chemists. Pomeranz will present his acceptance address at the AOAC 94th Annual meeting, scheduled at the Marriott Twin Bridges Hotel in Washington, DC, on Oct. 20–23, 1980. □

Appointments

Firmineich, Inc. has announced the appointments of **Peter Mueller** as account manager within the Flavor Division and **George MacDonald Birtwistle** as manager, marketing and evaluation, Flavor Division... **Halsey F. Smith** has been elected senior vice-president of equipment sales of Foster Wheeler Corporation and will also serve in the same position for the subsidiary, Foster Wheeler Energy Corporation... **David L. Shaw** has joined the engineering staff of EMI Corporation as project manager...Celanese Corporation has announced the election of **B.A. Bridgewater, Jr.**, as a member of its Board of Directors... **Frank J. Vitale** has joined Emery Industries Inc. as technical sales representative for the firm's Fatty and Dibasic Acids Group... Pfizer International has announced changes in its senior management: **Barry MacTaggart**, formerly executive vice-president of the company, has been named president; succeeding him as executive vice-president is **M. William Roche**, president of Pfizer Europe, who also was elected a vice-president of the parent company, Pfizer Inc; succeeding Roche as president of Pfizer Europe is **Robert Neimeth**, vice-president and director of operations of Pfizer Laboratories. Neimeth also has been named a vice-president of Pfizer International... **Charles H. Elster** has been named assistant corporate technical director of Lonza Inc. of Fair Lawn, NH, and **James Richardson** is now northwestern regional sales manager for the specialty chemical division of the firm... Norda Inc. has appointed **Duff Scott** perfumer in the corporation's fragrance division... Emery Industries, Inc. has promoted **Paul Van Nortwick** to product manager, Fatty and Dibasic Acids Group... **Louis E. Azzato** has been elected an executive vice-president of Foster Wheeler Corporation... Patco Products, a division of C.J. Patterson Co., Kansas City, MO, has named **James R. France** market development manager and **Fred Baiocchi** has become senior chemist in the food applications laboratory. Both assignments are in the company's food ingredients group.

de Vries

de Vries to manage Acidchem

Roy J. de Vries has been named general manager for Acidchem (M) Sdn Bhd, a fatty acid firm that is part of the Palmco Group of Malaysia. de Vries formerly was managing director of Alkaryl Chemicals (UK) Ltd.

Acidchem's fully integrated fatty acid and glycerine plant came on-stream during July. The plant will operate on palm, palm kernel and coconut oils, producing about 30,000 tons of fatty acids each year.

de Vries has worked in the fatty chemical industry

about 25 years, having previously worked with Armour in Europe, Canada and the United States before being named managing director of Alkaryl in 1974. □

DeBlanco promoted by Capital City

AOCS member John DeBlanco has been promoted to vice-president—manufacturing for Capital City Products Co., a division of Stokely-Van Camp Inc. DeBlanco will be directing a major expansion of the firm's facilities in Kearney, NJ.

DeBlanco has been operations manager for the firm's refinery in West New York, NJ, and manager of the firm's Kearny, NJ, manufacturing plant. He has been an AOCS member since 1973. □

Deaths

Evald L. Skau

AOCS has learned of the death of Dr. Evald L. Skau, a long-time member who joined the Society in 1939. He served 28 years as a research scientist with the Southern Regional Research Laboratory until he retired in 1967. Prior to this, he served 10 years as a faculty member of Trinity College in Hartford, CT. He received his B.S. and M.S. degrees from Trinity College in 1919 and 1920 and earned a Ph.D. from Yale in physical chemistry in 1925. Dr. Skau received the USDA's Superior Service Award in 1955 and the Honor Scroll from the American Institute of Chemists in 1975. Trinity College awarded him its Alumni Medal for Excellence in 1975.

Raymond A. Sunday

AOCS was recently notified of the death of Raymond A. Sunday. A member since 1959, Mr Sunday had a long career in management with several companies. He had been employed at Van Iderstine Co., H.M. Rubin Co., Inc., and Cape Fear Feed Products, Inc. His latest position was as general manager of Pine State By-Products, Inc., in South Portland, ME.

Lester Conrad

AOCS has been informed of the death of Lester I. Conrad, an AOCS member since 1962. Mr. Conrad had retired in 1977 from Amerchol, a unit of CPC International, Inc. He was a 1933 graduate of Brooklyn College and had been with Amerchol and its predecessor organizations since 1936, having served as technical director and vice-president for American Cholesterol Products, Inc.

Alan I. Fleischman

AOCS recently received notice of the death of Dr. Alan I. Fleischman. A member since 1965, Dr. Fleischman was a research scientist with the New Jersey State Department of Health. He received his B.S. degree from City College, New York, in 1950, his M.A. from Brooklyn College in New York in 1955 and his Ph.D. from St. John's University in New York in 1960.

Publications

Book reviews

Lipids: A Clinicians' Guide by L.A. Simons and J.C. Gibson (University Park Press, 233 E. Redwood St., Baltimore, MD 21202, 1980, 82 pp. $13.95).

Despite the all-encompassing title, this book deals only with coronary heart disease. The authors are proponents of risk factor intervention and believe in polyunsaturated fatty acids, restricted cholesterol intake, lipid-lowering drugs and ion exchange resins. They are, however, also reasonably objective, albeit a bit defensive, in their review of the literature. The multiple risk factor situation—hypertension, cigarette smoking and hyperlipidemia—usually has been viewed as posing the best opportunity for intervention. Simons and Gibson, however, focus solely on lipid intervention.

A few quotes from chapter III, "Is Risk Factor Intervention Worthwhile?" would seem appropriate. "Secondary prevention refers to management of patients who already have clinical manifestations of vascular disease. Secondary prevention has been practiced in trials and in clinical medicine for many years because such patients are numerous and have strong motivation. Secondary prevention intervention on behalf of lipids has been generally unrewarding (with certain exception to be discussed later in this chapter)." "Between 1965 and 1977 at least nine dietary intervention studies have been reported, four being primary prevention in type." "In essence, dietary intervention in the presence of existing coronary heart disease had no consistent effect on mortality although it may have reduced morbidity." "The Sydney Diet Heart Study offered a fat-modified polyunsaturated enriched diet to myocardial infarct survivors, aged 30 to 59 years, over a 2 to 7 year follow up period. Control subjects followed a diet with partial fat modification. Despite a favorable influence on plasma lipid levels there was no significant difference in survival between the two groups. This result essentially confirmed earlier findings in a U.K. study."

"The Coronary Drug Project was a double-blind trial in male survivors of myocardial infarction utilizing one of four therapeutic agents: estrogens, dextrothyroxine, nicotinic acid (niacin) and clofibrate. Excessive mortality in the active treatment group necessitated premature termination of patients on estrogens or thyroxine, but the six year experience with clofibrate and nicotinic acid was

reported in 1975. Neither drug had any significant influence on total mortality rates comparing active therapy with placebo, although a trend towards fewer non-fatal re-infarctions was observed in the nicotinic acid treated group." The following quotes refer to the WHO European Primary Prevention Trial with clofibrate. "This trial produced some disquieting features. The cholecystectomy rate for gallstones was significantly increased in the treated group, although still at a very low level. This effect has been described in previous clofibrate trials and in trials using polyunsaturated fats. Furthermore, there was an excess number of deaths in the active treatment group from diseases of gallbladder, liver and intestine, including malignant neoplasms of these sites."

If these direct quotes from two proponents of lipid intervention are indeed representative of the current state of the art, I hate to think how many millions of dollars have gone down the drain. The authors seem to express the view that the large trials have been unsuccessful because the extent of lipid lowering has been inadequate. They advocate aggressive treatment on an individual patient basis to provide a substantial decrement. In reading through the descriptions of the trials, however, one point consistently intrudes. Mortality does not seem to go down. What "side effects" are to be expected from such aggressive treatment?

As many in the fat and oil industry have learned in the last 25 years, it is necessary to be aware of recommendations being made to clinicians.

Lloyd A. Witting, Ph.D.
Supelco, Inc.
Bellefonte, PA 16823

Advances in Lipid Research, Vol. 17, edited by R. Paoletti and D. Kritchevsky (Academic Press, 111 Fifth Ave. New York, NY, April 1980, 308 pp., $31.00).

This is the latest volume of a well established review series published annually since 1963. Volume 16 was reviewed in this column in September 1979. Chapters include: Body Cholesterol Removal; Role of Plasma HDL, by Tall and Small; HDL Metabolism, by Nicoll, Miller and Lewis; Cholesterol Metabolism in Clinical Hyperlipidemias, by Sodhi, Kudchodkar and Mason; On the Mechanism of Hypocholesterolemic Effects of Polyunsaturated Lipids, by Paul, Ramesha and Ganguly; Lipid Peroxidation in Mitochondrial Membrane, by Vladimirov, Olenev, Suslova and Cheremisina; and Membrane Cooperative Enzymes As a Tool for the Investigation of Membrane Structure and Related Phenomena, by Farias. Two chapters on HDL seem appropriate and consistent with the tremendous recent interest in this area. Four chapters on cholesterol, however, appear excessive and certainly continues the trend in this series in recent years of over-emphasis in this area. The editors are to be particularly congratulated, however, on securing a good, readable chapter covering Russian work on lipid peroxidation and chemiluminescence. A great deal of this material has been previously available only in the Russian-language journals. There has been a definite surge of interest in chemiluminescence in the U.S. which should be further stimulated by appearance of this review. The final chapter focuses on membrane fluidity and allosteric enzymes. The role of membrane fluidity in the process of communication of hormonal action is extensively discussed.

As with previous volumes, the text is clear and the figures and tables are of good quality.

Lloyd A. Witting, Ph.D.
Supelco Inc.
Bellefonte, PA 16823

Nutrition and Environmental Health: The Influence of Nutritional Status on Pollutant Toxicity and Carcinogenicity, Vol. 1, The Vitamins, by E.J. Calabrese, (John Wiley and Sons, Inc., New York, 1980, 585 pp., $60.00).

This volume, part of the Wiley-Interscience series on Environmental Science and Technology, is the first of two designed to assess critically the influence of nutritional status on pollutant toxicity. The book contains five lengthy chapters, all of which are similarly organized. Each chapter has an introductory section that gives background on the vitamin. Subsequent sections are organized according to pollutant characteristics such as heavy metals, irritant gases, carcinogens and physical factors. The chapters end with a discussion and synthesis of the preceding sections.

Chapter 1 deals with vitamin A. There is an emphasis on vitamin A as a chemoprotective agent in hydrocarbon-induced epithelial cancer. The interactions of vitamin A with aflatoxin B, PCB, DDT, heavy metals, irritant gases, and radioactivity and noise are also covered.

The second chapter provides critical assessments of the interactions of the B vitamins with toxic substances in the environment. In addition to the types of toxic compounds considered with respect to vitamin A, the effects of various industrial compounds such as pyridine and hydrazine on individuals inadequate in B vitamins are considered. Also included is a major section on the effects of oral contraceptives on some B vitamins.

Ascorbic acid dietary status is the subject of chapter 3. This major chapter is divided into 10 sections as follow: background information on ascorbic acid; enzymatic detoxification; pesticides and herbicides; heavy metals; non-carcinogenic hydrocarbons; environmental carcinogens; interactions with medicines; air pollutant irritants; physical agents; and policy implications. The chapter ends with a discussion of the possible need to change the RDA for ascorbic acid because of its extensive interactions with a large number of pollutants.

Chapters 4 and 5 deal with vitamins D and E, respectively. The chapter on vitamin D considers its interaction with aflatoxin; the effect of D on the gastro-intestinal absorption of heavy metals; interactions with flouride and lead; ozone, vitamin D and the incidence of bone fractures; and other pollutant interactions. The chapter on vitamin E deals with similar pollutant interactions discussed with respect to vitamin A and the B vitamins but there is an emphasis on environmental oxidants such as ozone and nitrogen oxide.

This is a well organized book and the coverage is both comprehensive and critical. It raises a lot of questions about the possible need to modify the dietary intakes of vitamins in individuals exposed to certain pollutants. The book should be of value to those interested in environmental and occupational health and to nutritionists in general.

Patricia V. Johnston
Department of Food Science
University of Illinois
Urbana, IL 61801

New Products

XANTHOPHYLL FOOD COLORING

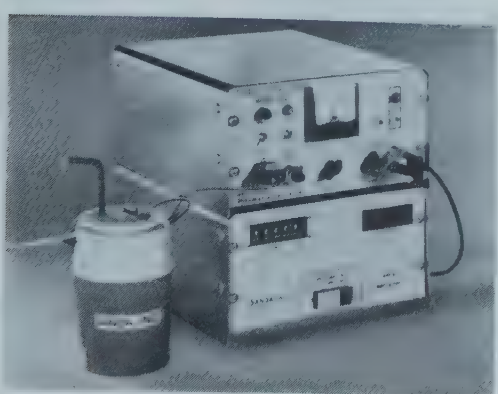
Burlington BioMedical Corporation is promoting a food coloring agent, Lutexan, to provide yellow color for food, pharmaceutical and cosmetic products. The substance is a natural xanthophyll extracted from alfalfa. It produces colors ranging from very light yellow to dark yellow. Vegetable oils, pastas, mayonnaise, salad dressing, butter, margarine and cheeses are among edible products in which it is suitable for use, the firm says. Contact: Burlington BioMedical Corporation, 100 Fairchild Ave., Plainview, NY 11803.

DIGITAL MICROMETER SYRINGES

Gilmont Instruments says its line of improved digital micrometer syringes and burets provide reading accuracies of one part in 25,000. Double 0-ring seals provide vacuum-tight joints and special gaskets confine fumes or liquid contact to teflon or glass only, the firm says. Capacities range from 0.25 to 2.5 ml with 0.00001- or 0.0001-ml vernier divisions, respectively. Contact: Gilmont Instruments Inc., 401 Great Neck Rd., Great Neck, NY 11021.

PIPETTING DISPENSER

Tri-Continent Scientific Inc. has introduced micro-set dispensers with volumes ranging from 10 ul to 100 ul, for use in pipetting small amounts of liquid. The less expensive model is made of polypropylene, glass and stainless steel; the more expensive model is made of inert fluorocarbon, glass and platinum-iridium. Contact: Tri-Continent Scientific Inc., 12541 Loma Rica Drive, Grass Valley, CA 95945.



TRACE ANALYZER FOR ACIDITY

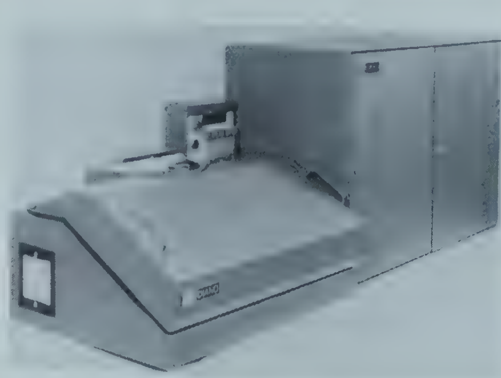
Sanda Inc. says its new automatic titrator will provide rapid and accurate analysis of minute amounts of acidity in fats and oils, rainwater and similar substances. The titration system consists of an electronic unit, a detector and a constant flow, digital buret. Contact: Sanda Inc., 4343 East River Dr., Philadelphia, PA 19129.

SINGLE-MULTIPOINT RECORDERS

International Products and Technologies Inc. says its 3122J series single and multipoint recorders can provide one continuous trace or 6– and 12–point sequentially printed multicolored-dot trace recordings. A patented magnetic balancing device makes it possible to trace-record on an 8–inch calibrated chart without cord drives or slide-wires, the firm says. Contact: International Products and Technologies Inc., 541 Davisville Rd., Willow Grove, PA 19090.

GAS, VAPOR DETECTION KIT

Scientific Gas Products' GD1000 gas and vapor detection kit consists of a pistol-type volumetric pump with direct reading detector tubes. Analysis is based on the chemical reaction of a sensitive reagent in the tubes to the gas being sampled. Detection tubes are available for more than 60 gases and solvent vapors. Contact: Scientific Gas Products Inc., 2330 Hamilton Blvd., South Plainfield, NJ 07080.



BENCH-TOP X-RAY SPECTROMETER

Diano Corporation says its new Chem-X spectrometer is designed for industrial use where elemental analysis is needed for production, process and quality control. The unit provides simultaneous analysis of up to eight elements. It identifies elements of atomic number 13 or higher at concentration ranges from parts per million to 100%. Contact: Diano Corporation, 30 Commerce Way, Woburn, MA 01801.

CENTRIFUGAL PUMP

Atlas Pumps' new Model 600-BK vertical cantilever shaft, corrosion-resistant, centrifugal pump is constructed in 316 stainless-steel. All bearings and discharge are above the mounting plate. The Model 600-BK is supplied with a 3 HP, 1750 rpm motor and with flexible coupling. Contact: Dept. RN, Atlas Pumps, 1703 East End Avenue, Chicago Heights, IL 60411.

SYNTHETIC LUBRICANT

Emery Industries Inc. has introduced Emgard universal SE-CD synthesized lubricant SAE-30, a universal synthetic lubricant for both gasoline and diesel engines. According to the company, the product is a premium lubricant that improves fuel economy, engine performance, low-temperature performance, oil and filter life span, engine cleanliness and corrosive wear resistance.

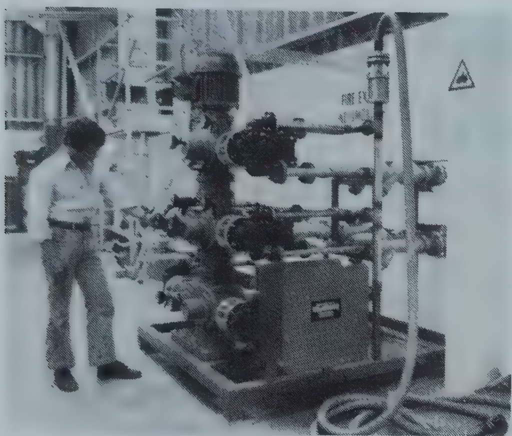
AUTOMATIC GC SYSTEM

Varian Associates Instrument Division has introduced an automated GC system, Vista 44, that combines the firm's Vista 401 Data System with up to four microprocessor-based Vista GC 4600 gas chromatographs. The 401 unit is used to establish setpoints and code and transmit them to the GC system, where the microprocessor automatically programs the correct parameters. The GC microprocessor transmits signals to the data system for real-time CRT status displays. Contact: Varian Associates, 611 Hansen Way, Palo Alto, CA 94303.

MARGARINE PACKAGER

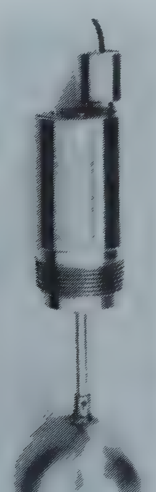
Lynch Machinery has introduced

Morpac TFC-76, a print, form and wrap machine designed primarily for margarines. Capacity is 4,500 pounds per hour in quarter-pound prints. Features include a variable speed drive motor with electrically controlled low speed start, a simplified mechanical power train, stainless steel forming head and covers with excess product recovery system. Contact: Lynch Machinery, 2300 Crystal St., Anderson, IN 46012.



LIQUID DETERGENT BLENDER

Bran & Lubbe has developed a multi-pumphead system to permit continuous blending of liquid detergent products. The unit uses eight pumpheads to which liquid raw materials are fed directly from storage tanks. Flow rates per pumphead may be up to 5,000 gallons per hour. Contact: Bran & Lubbe Inc., 1241 Rand Rd., Des Plaines, IL 60016.



LIQUID LEVEL CONTROL

Liquid Level Lectronics Inc. is offering its LV 471 series vertical mount electric high- or low-level control for sounding alarms, operating control equipment, or performing other electrical functions. The unit operates in any specific gravity of 0.7 or higher; it has a three-inch outside diameter sphere float. Unit price is $150. Contact: Liquid Level Lectronics Inc., PO Drawer 788, Porter, TX 77365.

PORTABLE WATER QUALITY MONITOR

Horiba's portable Model U-7 water quality monitor can be used to measure pH, dissolved oxygen, conductivity, temperature or turbidity. A sensor can be submerged in the water being tested or a small sample can be poured into a chamber within the sensor. Contact: Horiba Instruments, 1021 Duryea Ave., Irvine, CA 92714.

PNEUMATIC RELAYS

C-E Invalco offers two new models of pneumatic relays. CBS-2501-T1 models provide 1:1 input/output ratios and are designed to boost volume output of control devices to provide quicker response of low-volume output controls. CBS-2501-TS models provide 3:1 input/output. They may be used to boost volume and pressure output of controls with low pressure and volume output. Contact: C-E Invalco, Combustion Engineering Inc., PO Box 556, Tulsa, OK 74101.

NEW PRODUCT LITERATURE

Analabs, A Unit of Foxboro Analytical, 80 Republic Dr., North Haven, CT 06473, has recently published a wall chart, **Guide to Chromatographic Phases**, designed to help the chromatographer in selecting stationary phases... Whatman Inc., Paper Division, 9 Bridewell Place, Clifton, NJ 07014, is offering publication 824 GMF A, **Glass Microfibre Filters**, which discusses the characteristics and applications of these filters... Beckman Instruments, Inc., Process Instruments Division, Technical Information Section, 2500 Harbor Blvd., Fullerton, CA 92634, has a 14-page guide listing the company's complete line of process pH electrodes and thermocompensators... Hansen Manufacturing Co., 4050 W. 150th St., Cleveland, OH 44135, has issued an updated catalog for its line of quick-connective one-way shut-off couplings.... Vitec Inc., 23645 Mercantile Rd., Cleveland, OH 44122, has prepared a **Data Sheet for Model 642 Vibration Analyzer**... Anspec Co. Inc., PO Box 7044, Ann Arbor, MI 48107, has published a catalog featuring a variety of HPLC columns, TLC plates and sorbents manufactured by E. Merck, Darmstadt, Germany... Varian Associates, Inc., Industrial Equipment Group, 611 Hansen Way, Palo Alto, CA 94303, has a new 205-page **Catalog of Vacuum Components, Materials and Leak Detectors** available from regional Varian Industrial Product distribution centers... Babcock and Wilcox, PO Box 923, Augusta, GA 30903, has a four-page bulletin outlining the capabilities of its refractories and insulation.

Industry News

USDA tests pyridazione application to reduce linolenic acid

Two USDA scientists, Judith B. St. John and Meryl N. Christiansen, will attempt to reduce linolenic acid content in soy oil by applying pyridazione chemicals to the growing soybean plant. These chemicals have been demonstrated to block linolenic acid synthesis in the roots and leaves of several cereal crops and cotton. St. John and Christiansen, both researchers at the USDA Science and Education Administration facilities in Beltsville, MD, theorize that these chemicals might have the same effect in soybeans.

Soy oil, which accounts for about 40% of the value of the soybean crop, is at a competitive disadvantage in the market place because it contains a relatively high level of linolenic acid, a naturally occurring fatty acid which causes rancid flavor or spoilage of unprocessed oil. Presently, linolenic acid is reduced from 7% to an acceptable 3% by hydrogenation, but the process is costly.

The three-year study is being partially funded (about 60%) by a grant from the American Soybean Association Research Foundation (ASARF), totaling $45,000. ☐

Grindsted starts U.S. plant

Grindsted Products has begun production in its new Dimodan factory located at Industrial Airport, KS, 30 miles southwest of Kansas City, MO. The unit produces food-grade distilled monoglycerides, emulsifiers and derivatives.

The factory is the first major production unit outside Denmark for Grindsted's parent firm, Grindsted Products A/S. The firm produces food additives, vitamins and pharmaceuticals in Grenaa and Grinsted, Denmark. ☐

From Washington

Hearing set for Oct. 6 on revising food labeling

The Food and Drug Administration, U.S. Department of Agriculture and Federal Trade Commission have scheduled a public hearing for Oct. 6, 1980, on proposals to revise food labeling regulations.

The agencies are considering what information should be on labels, and in what form it should be printed. At the Oct. 6 meeting, a consultant will report on several alternatives and seek further suggestions. Three later meetings will be held (1) to review possible designs and discuss a proposed plan to test consumer reaction to specific proposals; (2) to discuss a specific consumer research plan; and (3) to discuss results of the consumer research plan and the labeling system that fared best in the consumer testing.

The entire program is expected to be completed by late 1981, according to the notice in the *Federal Register* of Tuesday, July 8, 1980, p. 45962.

Lead acetate deadline extended

The Food and Drug Administration has extended until Oct. 31, 1980, its self-imposed deadline for deciding whether to permit the continued use of lead acetate as a color additive for cosmetics applied to hair on the scalp. Details: *Federal Register,* Tuesday, June 24, 1980, p.42255.

Tallow to be used in fabric softener

Grestco-Dyes & Chemicals Inc. of Thomasville, NC, has filed a premanufacture notice with the federal EPA to produce a fabric softener for use on knitted and woven fabrics for industrial use. The softener's chemical identity as listed in the notice is "amide from diethylenetriamine and tallow ester compounds with diethylsulfate." Details: *Federal Register*, Wednesday, May 7, 1980, p. 30130.

Tolerance extended for cotton insecticide

The EPA has renewed until May 8, 1981, the temporary tolerance for residues of the insecticide oxamyl (methyl-N,N dimethyl-N-[methylcarbamoyl)oxy] -1-thiooxamimidate in or on cottonseed at 0.2 part per million (ppm). The renewal was requested by E.I. du Pont de Nemours & Co. Details: *Federal Register,* June 13, 1980, p. 40219.

Codex fats and oils compendium to be published

The Codex Alimentarius Committee on fats and oils plans to publish a loose-leaf compendium of fats and oils standards developed by the committee. The standards previously have been available as separate booklets for each standard. The loose-leaf format will permit easy inclusion of supplements, revisions and additions as they become available.

A.W. Hubbard, who has served as chairman for the committee for a number of years, retired at the end of the committee's June 1980 meeting. The committee approved a resolution offered by the U.S. delegation commending Hubbard and thanking him for his service.

The following report on the meeting was prepared by Dr. R.J. Sims, the AOCS Representative to the Codex Fats and Oils Committee.

CODEX COMMITTEE ON FATS AND OILS, ELEVENTH SESSION, LONDON, JUNE 23-27, 1980

Delegates from 30 countries and observers from 13 international organizations attended the session. This committee's function is to develop standards for edible fats and oils which will insure their identity and quality to the consumer. These standards also should facilitate international trade in vegetable oils and margarine.

General Standard for Fats and Oils

Most oils moving in world trade require further processing to make them suitable for human consumption. But the standard as presently written covers only edible oil which does not require additional processing. The U.S. delegation suggested that, since the identity characteristics (especially fatty acid ranges by GLC) of the individual oils were also applicable to crude oils, the general standard be extended to include these crude articles of commerce. This identity characteristic, but not quality criteria, would apply to bulk shipments which required further processing before consumption. There was a consensus that such an extension was desirable but the committee was divided on how the change should be incorporated into the Codex standards. Governments will be asked to comment on the proposals.

Reduced-fat Margarine

The committee agreed that two standards are needed for reduced-fat margarine. The more restricted one applies to Minarine containing 39-41% fat which is marketed widely in Europe. The Minarine standard was advanced to step 8 of the 10-step Codex procedure. It may be designated by some name other than Minarine in accordance with the laws and customs of the individual countries. One unsettled issue is the list of permitted additives which must first be reviewed by the Codex Food Additives Committee.

A second standard to cover products other than "Minarine" provoked considerable discussion. There was no general agreement on what ranges of fat content should be covered. The U.S. delegation favors an upper limit of 60% but no lower limit. It was agreed to advance the standard to step 5 so that it could be considered by the commission at its meeting in 1981.

GLC Identification Based on Fatty Acid Ranges

A collaborative study carried out by Dr. W.H. Tallent of USDA, Peoria, IL, and Dr. J.P. Wolff of ITERG, Paris, demonstrated that there is a compatibility between the fatty acid ranges based on GLC and iodine values, refractive indices and saponification values. European laboratories favor use of classical methods of identification since they regard GLC as less than completely satisfactory for detection of adulteration in many cases. The olive-growing countries particularly are concerned with this problem and are attempting to introduce identity characteristics based on sterol ranges for all oils. Additional developments in analytical methods for sterols are needed before such ranges can be defined. The GLC graphic procedure was published in this journal (*JAOCS* 56:972).

Commercial Processing of Fats and Oils and Processing Aids

There was general agreement that a code of practice for processing fats and oils and the list of processing aids should not be mandatory since otherwise technological development would be inhibited. There appeared to be little enthusiasm for developing such a code of practice, but there was some concern about establishing residual levels of processing aids and any toxicological problems which might result. Data on residual levels are often lacking but generally levels are very low so that the problem should be minimal. If the residual level of the material is below that required to perform a technological function, then it need not be listed as a food additive. The list of processing aids is purely advisory and an open one at this stage. It will contain a summary of information on typical residual levels received from each government and the acceptable daily intake, if available.

The committee also considered the development of standards for vegetable and animal ghees and mixtures thereof. There was a discussion relating to amending the standard for rapeseed oil. These standards (ghee and rapeseed oil) were advanced to step 3 of the Codex procedure.

The secretariat will prepare a compendium of fats and oils standards developed by the committee to be included in a single book. This will be of loose-leaf type so that supplements, revisions and additions can be included when they become available.

The committee will meet again in London in 1982 or 1983. □

World Conference on SOYA Processing and Utilization

CENTRO ACAPULCO • ACAPULCO, MEXICO November 9-14, 1980

120 specialists to speak

Registrants at the World Conference on Soya Processing and Utilization will participate in one of the most intensive one-week programs ever held on soya.

By the end of the week of Nov. 9-14, 1980, more than 120 plenary and round table speakers will have provided a thorough review of soybeans—from how researchers are working to increase the quantity and quality of soybeans being grown, to how to crush the soybeans into oil and meal, through how oil is processed into edible products and how meal is used in its traditional role as animal feed as well as its growing role as a source of protein for humans. The conference will be held in Acapulco, Mexico.

The conference is designed to transmit state-of-the-art knowledge and techniques about the processing and use of soya, particularly for nations that could improve the nutritional quality and quantity of foods in their diet through a sustained growth in the use of soya. The meeting will provide a means to establish constructive and continuing dialogs between experts in soya products and processing and those professionals who can put such knowledge to practical use.

As of early August, the plenary program had been completed: 54 speakers from North America, Latin America and Japan are scheduled to speak. The plenary program is divided into 6 general subject areas: Production; Processing; Effects of Processing on Soya Oil—Analytical, Physical Characteristics and Nutrition; Soya Meal in Animal Feeds; Soya Protein for Human Foods; Preparation, Characteristics and Uses; and Soya Protein for Human Foods: Nutritional and Regulatory Aspects.

The plenary sessions will be held each morning with simultaneous translation of English and Spanish. During early afternoons, there will be an exposition in the Acapulco Centre. Informal round table discussions will be held each day from about 3 p.m. to 6 p.m. Approximately 65 speakers have been confirmed for these sessions. These speakers will give brief papers providing more detail of a plenary topic, or perhaps providing additional general information. Most of the discussion group time is reserved for registrants to question speakers.

In addition to the program at the center, registrants may participate in field trips to nearby soya growing regions. Acapulco is located in the state of Guerrero in Mexico. Guerrero has for many years been engaged in an extensive research program to improve soya production as a means of improving nutritional value of local diets.

Besides the formal plenary, exposition, round table and field trip portions of the program, organizers have provided ample opportunity for informal discussion among registrants and speakers. There will be an informal reception Sunday evening, Nov. 9, to mark the opening of the exposition. On Monday evening, Nov. 10, there will be an outdoor reception at the Acapulco Centre. On Friday, Nov. 14, there will be a gala social event for all registrants. There will be 2 sponsored lunches for conference registrants with soya foods available.

Registration materials are available from the AOCS, 508 S. Sixth St., Champaign, IL 61820. Registration may be completed on site. Housing reservations should be made through Excursions Unlimited, Inc., 1820 Northwest Boulevard, Columbus, OH 43212, USA. □

Call for papers— AOCS 1981

The Technical Program Committee has issued a call for papers to be presented during the 72nd annual meeting of the American Oil Chemists' Society to be held May 17-21, 1981, at the Fairmont Hotel, New Orleans, Louisiana. Papers on every aspect of lipids, oils, fats and related areas are welcome. Please submit three copies of a 100-to-300-word abstract with title, speaker and co-authors clearly indicated. Presentations normally are 20 minutes in length. Please also indicate if you wish to make the presentation in the normal lecture manner or in a poster session. The abstracts are to be mailed to: Dr. Thomas Jacks, 1981 Technical Program Chairman, USDA Southern Regional Research Center, PO Box 19687, New Orleans, Louisiana 70179 USA.

Deadline: December 1, 1980

□

FOR OFFICE USE ONLY

Registration # ______ Total Fee ______ Balance due ______

Amount received ______ Room number ______

World Conference on SOYA Processing and Utilization

CENTRO ACAPULCO • ACAPULCO, MEXICO
November 9-14, 1980

REGISTRATION FORM • FORMA DE INSCRIPCION

AOCS World Soya Conference — 508 S. Sixth Street — Champaign, Illinois 61820 — USA

IMPORTANT: Before you complete this form, read the conditions on the back.

IMPORTANTE: Antes de llenar esta forma, lea las condiciones al reverso.

PLEASE USE A TYPEWRITER • POR FAVOR UTILICE UNA MAQUINA DE ESCRIBIR

DELEGATE
DELEGADO

Family Name
Apellido(s) ______ First Name
Nombre ______

Nickname
Apodo ______

Employer
Compañía ______

Address
Dirección ______ City
Ciudad ______

State
Estado ______ Country/Postal Code
País/Zona Postal ______

Telephone
Teléfono ______ Telex
Telex ______

PAYMENT PAGO	Price per Person Precio por Persona			
	Before Antes	10 Oct	After Después	Total
Registration Fee Cuota de Inscripción	$300 US		$350 US	
Ladies' Program Programa de Damas	$100 US		$125 US	
	GRAND TOTAL SUMA TOTAL			

The total amount is remitted by:
La cantidad total ha sido remitida por:

☐ Bank Transfer to:
Transferencia Bancaria a:
AOCS World Conference
Account Nr. 10-267-3
508 S. Sixth Street
Champaign, IL 61820 USA

☐ Enclosed Check
Cheque adjunto

Ladies' Program Participant
Participante del Programa de Damas

Family Name
Apellido ______ First Name
Nombre ______

Signature
Firma ______

Place and Date
Lugar y Fecha ______

AMERICAN OIL CHEMISTS' SOCIETY

GENERAL CONDITIONS

1. Registrations are not official and complete until payment is made.
2. A copy of this form will be returned to confirm your registration. Please bring your copy to the conference.
3. Use a separate form for each delegate. Payment can be combined for more than one registrant when forms are sent in together.
4. The committee requests that all payments be made in U.S. Dollars.
5. Cancellations *received* prior to October 10, 1980, will be refunded in full. Cancellations between October 10 and November 7 will be assessed a reasonable penalty to cover expenses incurred by the conference. Cancellations received after November 7 will not be refunded unless special exceptions are made by the committee.

CONDICIONES GENERALES

1. Las inscripciones no son oficiales ni completas hasta hecho el pago.
2. Una copia de esta forma le será devuelta confirmando su inscripción. Por favor traiga la copia a la conferencia.
3. Utilice una forma para cada delegado. El pago puede ser combinado para varios participantes si las formas son enviadas juntas.
4. El comité solicita que todos los pagos se hagan en dólares americanos.
5. Las cancelaciones recibidas antes del 10 de Octubre del 1980, serán reembolsadas en su totalidad. Aquellas hechas entre el 10 de Octubre y el 7 de Noviembre tendrán un cargo razonable de acuerdo a los gastos en que se haya incurrido. Las cancelaciones recibidas después del 7 de Noviembre no serán reembolsadas a menos que el comité asï lo autorice.

WORLD CONFERENCE ON SOYA PROCESSING AND UTILIZATION

CANCELLATION AND REFUND POLICY

The participant has the right to cancel his reservation and receive a refund as set forth in the following schedule. All requests for refund must be sent in writing or by telegraphic notice to Excursions Unlimited, Inc., 1820 Northwest Blvd., Columbus, Ohio, 43212

If your notice is received:	You will receive:
61 or more days before departure.	Refund of deposit less an administrative fee of $25.00 per person.
At any time within 60 days before departure, if air seat and hotel room are resold.	A full refund, less a $25.00 per person administrative fee.
At any time within 30 days before departure.	You are subject to a $100.00 per person cancellation charge plus any additional charges assessed by the hotels, airlines and ground service operators.

DISCLOSURE STATEMENT

These trips are operated by Excursions Unlimited, Inc. All tickets and coupons covering transportation, hotel accommodations or other services and facilities furnished are issued by them only as agent for such other companies furnishing such services and facilities and neither they nor their sub-agents shall be held liable for loss or damage, or injury to property, or injury to person, caused by reason of any defect by any transportation company, hotel agent, or any such party providing services. They reserve the right to withdraw any tour in this program at any time and to make changes and alterations in the itineraries as may be found necessary for the proper handling of said tours. The Airlines concerned are not to be held responsible for any act, omission or event during the time that passengers are not on board their planes or conveyances. The passage contract in use by the Airlines concerned, when issued, shall constitute the sole contract between the Airlines, and the purshasers of these tours and/or passengers. Neither Excursions Unlimited nor their sub-agents shall be held responsible for loss of or damage to baggage. Baggage insurance is recommended and can be obtained through Excursions Unlimited, Inc.

The American Oil Chemists' Society, its officers and employees assume no responsibility for any personal injuries or damage to property incurred by a member or guest of said conference sponsored activity.

Excursions Unlimited, Inc., Travel/Convention Consultants, has been officially designated as the Tour Producer by The American Oil Chemists' Society to organize, offer and coordinate the Conference programs offered in conjunction with the World Conference on Soya Processing and Utilization.

OFFICIAL TRAVEL & HOTEL RESERVATION FORM
WORLD CONFERENCE ON SOYA PROCESSING & UTILIZATION
ACAPULCO – NOVEMBER 9 - 14, 1980

Please complete and return with deposit to: Excursions Unlimited, Inc., 1820 Northwest Boulevard, Columbus, Ohio 43212.

PLEASE PRINT OR TYPE **PLEASE SUBMIT ONE FORM PER COUPLE AND/OR FAMILY**

Name ____
Last First Spouse's First Name (if accompanying)

Child's Name (if accompanying) ____________ Age ___ / ____________ Age ___

Billing Address ____

City ____________ State ____________ Zip ____________ Bus. Phone ____________

Home Address ____

City ____________ State ____________ Zip ____________ Home Phone ____________

PREFERRED PROGRAMS:

_______ Program A Conference Only - Acapulco November 8 - 15, 1980

_______ Program B Post Conference to Cancun November 15 - 18, 1980

_______ Program C Post Conference to Mexico City November 15 - 18, 1980

HOTEL PREFERENCE IN ACAPULCO
(number 1st, 2nd, 3rd choice):

_______ Hyatt Regency (Conference Headquarters)
_______ Condesa Del Mar
_______ El Presidente
_______ Fiesta Tortuga
_______ La Palapa

PREFERRED ACCOMMODATIONS:

_______ Single (1 person to a room)
_______ Double (2 persons to a room, 1 double bed (on request)
_______ Twin (2 persons to a room, twin beds)
_______ Triple (3 persons to a room)

Please write Excursions Unlimited for hospitality suite information.

AIR TRANSPORTATION

I/We desire: ☐ No air arrangements required
☐ Air transportation requested
☐ Smoking ☐ Non-Smoking

I/We will depart from ____________ on ____________
City/State date

and return to ____________ on ____________
City/State date

Preferred air service:
☐ First Class
☐ Coach Class
☐ Individual Tour Basing

Special Requirements:
I/We have special requirements for days of travel.
Please book my airline reservations as follows:

From ____________ to ____________ on ____________
City City Date

From ____________ to ____________ on ____________
City City Date

From ____________ to ____________ on ____________
City City Date

If additional space is required please use the reverse side of this form.

RESERVATION DEPOSIT INFORMATION

_____ Conference Only @ $150.00 per person = _______

_____ Post Conference Program to Cancun @ $50.00 per person = _______

_____ Post Conference Program to Mexico City @ $50.00 per person = _______

Total Advance Deposit Due: _______

Check or Money Order Enclosed (Make payable to Excursions Unlimited, Inc.) I prefer to pay my deposit by:

Master Charge/Visa (circle one)
Card No. ____________ Exp. Date ________
Signature ____________________

I have read the information on deposit payments and cancellation policy as stated in the brochure.

Signature ______________________________ Date ____________

WORLD CONFERENCE TRAVEL PLANS

PROGRAM A — CONFERENCE ONLY — ACAPULCO
November 8 - 15, 1980

Inclusive Features:

- Hotel accommodations for eight (8) days and seven (7) nights in Acapulco.
- Local hotel tax.
- Pre-registration of guests at hotel.
- Special Acapulco Bay Sunset Cruise including round trip hotel/pier transportation.
- Round trip transportation between airport and hotel in Acapulco.
- Local Mexican Escorts, including gratuities.
- Services of Excursions Unlimited Senior Staff Members.
- Baggage handling at the airport and hotel upon arrival and departure.

PACKAGE COST EXCLUSIVE OF ROUND TRIP AIR FARE FROM SELECTED CITY:

	Per Person Sharing Twin	Single Supplement	Per Person Triple Reduction
Hyatt Regency (Conference Headquarters)	$267.00	+$234.00	-$20.00
Condesa Del Mar	$267.00	+$234.00	-$20.00
El Presidente	$240.00	+$193.00	-$10.00
Fiesta Tortuga	$214.00	+$157.00	-$10.00
La Palapa	$214.00	+$157.00	-$20.00

PROGRAM B — ACAPULCO CONFERENCE AND POST-CONFERENCE TO CANCUN
November 8 - 18, 1980

Inclusive Features:

- Hotel accommodations for eight (8) days and seven (7) nights in Acapulco.
- Hotel accommodations for four (4) days and three (3) nights at the EL PRESIDENTE Hotel, Cancun.
- Local hotel tax.
- Pre-registration of guests at hotels.
- Special Acapulco Bay Sunset Cruise including round trip hotel/pier transportaton.
- Round trip transportation between the airports and hotels upon arrival and departure in Acapulco and Cancun.
- Baggage handling at the airports and hotels upon arrival and departure in Acapulco and Cancun.
- One half-day tour to Tulum, the only walled Mayan City, Cancun.
- Local Mexican Escorts, including gratuities.
- Services of Excursions Unlimited Senior Staff Members.

COST OF POST ONLY INCLUDING ONE-WAY AIR TRANSPORTATION ACAPULCO/CANCUN

Per Person Sharing Twin	Single Supplement	Per Person Triple Reduction
$331.00	+$117.00	-$10.00

PROGRAM C — ACAPULCO CONFERENCE AND POST-CONFERENCE TO MEXICO CITY
November 8 - 18, 1980

Inclusive Features:

- Hotel accommodations for eight (8) days and seven (7) nights in Acapulc
- Hotel accommodations for four (4) days and three (3) nights.
- Local hotel tax.
- Pre-registration of guests at hotels.
- Special Acapulco Bay Sunset Cruise including round trip hotel/pier transportation.
- Round trip transportation between the airports and hotels in Acapulco and Mexico City.
- Baggage handling at the airports and hotels upon arrival and departure in Acapulco and Mexico City.
- One half-day tour to the Pyramids and Shrine of Guadalupe, the great religious center of the Teotihuacan civilizations.
- Local Mexican Escorts, including gratuities.
- Services of Excursions Unlimited Senior Staff Members.

COST OF POST ONLY INCLUDING ONE-WAY AIR TRANSPORTATION ACAPULCO/MEXICO CITY

Per Person Sharing Twin	Single Supplement	Per Perso Triple Reductio
$180.00	+$87.00	-$10.00

SAMPLE AIRFARES

(The following sample round trip airfares are quoted in order to illustrate the difference between regular airfares to Acapulco and those available to registrants using the travel/housing packages to be provided by Excursions Unlimited. These fares were based on schedules in effect in late 1979 and, because of anticipated price increases due to rising fuel costs and other factors, probably are lower than the prices that will be in effect in November 1980 at the time of the conference. They are quoted for comparative purposes only; please consult Excursions Unlimited for more up-to-date prices.)

Chicago round trip to Acapulco as part of tour, $237; regular round trip coach fare, $452.

New York round trip to Acapulco as part of tour, $303; regular round trip coach fare, $518.

Los Angeles round trip to Acapulco as part of tour, $248; regular round trip coach fare, $290.

Dallas round trip to Acapulco as part of tour, $194; regular round trip coach fare, $292.

Oilseed meeting in Buenos Aires

The VII Simposio Nacional and the IV Latinamericano de Oleaginosos conference will be held Nov. 17–19, 1980, in the Bolsa de Cereales headquarters in Buenos Aires, Argentina.

Past meetings have attracted representatives of the producers, processors and users of oilseed products. Details are available from: Oficina de Coordinacion, Correintes 127 - 2° Piso, Buenos Aires, Argentina. □

Oil palm meeting in 1981

An international conference covering all aspects of oil palm from breeding through final product usage has been tentatively scheduled for June 17–20, 1981, in Kuala Lumpur. Sponsors will be The Palm Oil Research Institute of Malaysia and the Incorporated Society of Planters.

Topics will range from development of new varieties, through agronomic practices, pest and disease control, harvesting, processing, effluent treatment, processing of oil, products and their uses, and economics and marketing.

An initial circular on the conference is available from the Conference Secretary, c/o Incorporated Society of Planters, PO Box 262, Kuala Lumpur 01-02, Malaysia. People wishing to present papers on a relevant topic should indicate that topic. Potential attendees also should indicate if they would be interested in visiting plantations and refineries, research institutes, or refining, bulking and shipping complexes. □

Call for papers

The Second World Congress of Chemical Engineering and World Chemical Exposition, "Chemical Engineering for World Development," Oct. 4–9, 1981, in Montreal, Canada, will be cosponsored by the Interamerican Confederation of Chemical Engineering, European Federation of Chemical Engineering and Asian Pacific Confederation of Chemical Engineering. Subjects include: economic and technological outlooks, international cooperation, energy development and utilization, reaction engineering, environmental chemical engineering, polymer engineering, biomedical engineering and biochemical engineering. Name, address and five copies of a 300-word abstract must be submitted to the Congress Secretariat by Oct. 1, 1980. For information write: Congress Secretariat, 2nd World Congress of Chemical Engineering, 151 Slater St., Suite 906, Ottawa, Ontario, Canada K1P 5H3. □

Local Sections

Pelick receives Northeast award

The AOCS Northeast Section presented its 1980 Professional Service Award to Nicholas Pelick, president of Supelco Inc., at the group's opening meeting of the fall season. The meeting was held Sept. 9 at the Robin Hood Inn in Clifton, NJ.

Pelick spoke on current and future trends in chromatography, with emphasis on triglyceride analysis using both gas liquid chromatography and thin layer chromatography.

A veteran, active member of the Northeast Section, Pelick also has served on the AOCS Governing Board and numerous AOCS committees, including Biochemical Methods, Education and Award in Lipid Chemistry. He was the recipient of the AOCS Award of Merit in 1978. Pelick also is chairman of the AOCS Foundation committee. He spoke briefly to the Northeast Section on the Foundation proposal to compile international fats and oils methods into a single publication.

Pelick also served as chairman of a speakers' fund-raising committee that solicited contributions from industry to help defray costs of foreign speakers attending the recent ISF/AOCS World Congress in New York City. □

Obesity symposium to be December 9

The AOCS Northeast Section will hold its annual one-day symposium on Tuesday, Dec. 9, 1980, featuring a series of papers on obesity and its relationship to dietary fat.

General chairman is Mark Bieber, senior nutritionist at Best Foods Division of CPC International in Union, NJ. Program chairman is David Kritchevsky of The Wistar Institute in Philadelphia.

The tentative location selected is the Howard Johnson's facility in Saddlebrook, NJ, but final arrangements had not been completed as of late July. Program details as well as registration material and final arrangements will be published in the October *JAOCS*. Northeast Section members will receive details and registration forms in late October or early November. Other persons wishing to have information mailed to them should write to Obesity and Dietary Fat, AOCS, 508 S. Sixth St., Champaign, IL 61820 USA. □

Calls for nominations

AWARD IN LIPID CHEMISTRY

In April 1964, the Governing Board of the American Oil Chemists' Society established an Award in Lipid Chemistry under the sponsorship of the Applied Science Laboratories, Inc., State College, PA. Previous awards were presented as follows: Erich Baer, 1964; Ernest Klenk, 1965; H.E. Carter, 1966; Sune Bergström, 1967; Daniel Swern, 1968; H.J. Dutton, 1969, E.P. Kennedy, 1970; E.S. Lutton, 1971; A.T. James, 1972; F.D. Gunstone, 1973; P.K. Stumpf, 1974; W.O. Lundberg, 1975; George Popjak, 1977; Ralph Holman, 1978; Stephen S. Chang, 1979; and James F. Mead, 1980.

The award consists of $2,500 accompanied by an appropriate certificate. It is planned that the seventeenth award will be presented at the AOCS Annual Meeting in New Orleans, May 17-21, 1981.

Canvassing Committee Appointees

Policies and procedures governing the selection of award winners have been set by the AOCS Governing Board. An Award Nomination Canvassing Committee is appointed; chairman is Earl G. Hammond. The function of this committee is to solicit nominations for the seventeenth award. Selection of the award winner will be made by the Award Committee whose membership will remain anonymous.

Rules

The rules prescribe that nominees will have been responsible for the accomplishment of original research in lipid chemistry and must have presented the results thereof through publication of technical papers of high quality. Preference will be given to individuals who are actively associated with research in lipid chemistry and who have made fundamental discoveries that affect a large segment of the lipid field. For award purposes, the term "lipid chemistry" is considered to embrace all aspects of the chemistry and biochemistry of fatty acids, of naturally occurring and synthetic compounds and derivatives of fatty acids, and of compounds that are related to fatty acids metabolically or occur naturally in close association with fatty acids or derivatives thereof. The award will be made without regard for national origin, race, color, creed, or sex.

Letters of nomination together with supporting documents must be submitted in octuplicate to Earl G. Hammond, Department of Food Technology, Iowa State University, Ames, IA 50011, USA, before the deadline of November 1, 1980. The supporting documents will consist of professional biographical data, including a summary of the nominee's research accomplishments, a list of his publications, the degrees he holds, together with the names of the granting institutions, and the positions held during his professional career. There is no requirement that either the nominator or the nominee be a member of the American Oil Chemists' Society. In addition, letters from at least three other scientists supporting the nomination must be submitted in octuplicate.

Remember the deadline: November 1, 1980

ALTON E. BAILEY AWARD

The North Central Section of AOCS is requesting written nominations from Society members for the 1979-80 Alton E. Bailey Award. The purpose of the Bailey Award is to recognize research and/or service in the field of fats and oils. The nomination should contain at least five pertinent references or contributions in the field of oils, fats, waxes, etc. Some of the past Bailey Award winners are: V.C. Mehlenbacher, 1959; R.H. Potts, 1960; J.C. Cowan, 1961; A.R. Baldwin, 1963; T.P. Hilditch, 1965; D. Swern, 1966; W.O. Lundberg, 1967; H.J. Dutton, 1968; H.S. Olcott, 1969; H.E. Carter, 1970; J.F. Mead, 1971; R.T. Holman, 1972; C.M. Gooding, 1973; S.S. Chang, 1974; W.M. Cochran, 1975; Raymond Reiser, 1976; L.A. Goldblatt, 1977; O.S. Privett, 1978; R.O. Feuge, 1979; Frank Norris, 1980.

Please send nominations to the Alton E. Bailey Award Chairman, Frank R. Kincs, Bunge Edible Oil, 1919 Swift Drive, Oak Brook, IL 60521; phone (312) 325-9320. The deadline for nominations is December 15, 1980, and notification of the selection will appear in this journal. The presentation of the Bailey Award is scheduled for early 1981.

Deadline: December 15, 1980

1981 HONORED STUDENT AWARDS

Nominations are now being solicited for the 1981 AOCS Honored Student Awards. Graduate students at any North American institution of higher learning, in any area of science dealing with fats and lipids, who are doing research toward an advanced degree, and who are interested in the areas of science and technology fostered by this Society, are eligible. To receive the award, he/she must remain a registered graduate student and must not have received his/her degree or begun career employment prior to the AOCS meeting he/she is to attend. Selection of awardees is based upon educational qualifications and performance.

The awards provide funds equal to travel costs, plus an additional stipend to permit attendance at the national meeting of the AOCS to be held in New Orleans, May 17-21, 1981.

Nomination forms may be obtained from AOCS Headquarters, 508 S. Sixth St., Champaign, IL 61820.

Deadline: October 20, 1980

Official Referee Chemists

The AOCS Examination Board has certified Official Referee Chemists in the laboratories listed below. The laboratories are listed in alphabetical order. Each laboratory has been assigned a number code; each analytical category has been assigned a letter code. The two lists are cross-referenced. Each laboratory listing includes the letter code for analytical categories in which it has an Official Referee Chemist; each category listing includes the number code for those laboratories employing Official Referee Chemists for that category.

1. A&L Plains Agricultural Laboratories
707 Avenue "H"
PO Box 1590
Lubbock, TX 79408
Arthur W. Carnrick
W. Avery Fix
(A, E, N,)

2. Abed Laboratories
2437-6th St.
Berkeley, CA 94710
Mamdouh H. Abed
(F, I)

3. Agri Science Laboratories, Inc.
16633 Valley View Ave.
Cerritos, CA 90701
Kenneth P. Stoub
(D, N)

4. Alabama Testing Laboratories
PO Box 10881
Birmingham, AL 35202
James C. Lloyd
(A, D)

5. Arizona Testing Laboratories
817 W. Madison St.
Phoenix, AZ 85007
Claude E. McLean, Jr.
(A, N)

6. Associated Laboratories
1815 W. Chapman Ave.
Orange, CA 92668
Henry M. Espoy
(D, I)

Bacon Laboratories
(See Charles V. Bacon, Inc.)

7. Barrow-Agee Laboratories, Inc.
405 Saturn Dr.
PO Box 156
Memphis, TN 38101
Lynn A. Hawkins
Travis Maxwell
Cecil B. Speer
Edgar H. Tenent
(A, C, D, F, I, K, L, M)

8. Barrow-Agee Laboratories of Mississippi, Inc.
837 O'Hea St.
PO Box 771
Greenville, MS 38702
S. Wallace Graves
(A, D)

9. Barrow-Agee Laboratories of Mississippi, Inc.
870 Foley St.
PO Box 2551
Jackson, MS 39207
Plautus L. Phillips
(A, C, D, F)

10. Caleb Brett U.S.A., Inc.
1406 - 26th St.
PO Box 441
Kenner, LA 70063
Harvey Becnel
(C, D, F, I, L, M)

11. Central Analytical Laboratories, Inc.
2600 Marietta Ave.
PO Box 188
Kenner, LA 70062
Leon J. Cabes, Jr.
(D)

12. Charles V. Bacon, Inc.
PO Box 441
Lansing, IL 60438
Dean F. Yingling
(I)

13. Charles V. Bacon, Inc.
730 Barataria Blvd.
PO Box 97
Marrero, LA 70072
Ramesh Patel
Albert M. Reynaud
(D, F, I)

14. Charles V. Bacon, Inc.
34 Exchange Place
Jersey City, NJ 07302
Charles V. Bacon, Jr.
Richard W. Martin
T.M. Narayanan Nair
(F, I, L)

15. Charles V. Bacon, Inc.
1308 Holland Ave.
Galena Park, TX 77547
Elmer Brinkley
(D, F, I)

16. Curtis & Tompkins, Ltd.
290 Division St.
San Francisco, CA 94103
Betty L. Miller
(D, G, I)

17. Deep South Laboratory
PO Box 4133
Montgomery, AL 36104
Charles R. Jenkins
(A, B, C, D, F)

18. Dickinson Laboratories, Inc.
PO Box 10057
El Paso, TX 79992
George G. Dickinson
(E, G)

19. Fox Testing Laboratories, Inc.
201 Paris
PO Box 1299
Lubbock, TX 79408

Paul D. Cretien
Ronald M. Fox
(A, B, D, F, J)

20. Geo. W. Gooch Laboratories, Ltd.
1250 Boyle Ave.
Los Angeles, CA 90023
Robert M. Gilpin
Roger C. Miller
(D, H, I)
Gooch Laboratories
(See Geo. W. Gooch Laboratories, Ltd.)

21. Hahn Laboratories
1111 Flora St.
PO Box 1177
Columbia, SC 29202
Edward R. Hahn
James B. Scoggins
(A, B, C, D, F, I, J, K)

22. Houston Laboratories
516 Arlington St.
PO Box 132
Houston, TX 77001
Jesus A. Garcia
(A)

23. Industrial Laboratories Co., Inc.
1450 E. 62nd Ave.
PO Box 16207
Denver, CO 80216
Maurice A. Rust
(I)

24. Industrial Laboratories
3001 Cullen St.
Fort Worth, TX 76107
Donald C. Strathdee
(D, O)

25. Ingman Laboratories, Inc.
324 Fourth Ave.
PO Box 15303
Minneapolis, MN 54415
Glenn H. Kyle
Philip L. Maiers
(C, D, I)

26. Iowa Testing Laboratories, Inc.
PO Box 188
Eagle Grove, IA 50533
Jack W. Henry
(D)

27. Japan Food Research Laboratories
52-1, Motoyoyogi, Shibuya-ku
Tokyo, Japan
Minoru Saito
(I)

28. Jennings Laboratories
118 Cypress Ave.
PO Box 851
Virginia Beach, VA 23451
William H. Jennings
William H. Jennings, Jr.
(C, D, K, M)

29. K-Testing Laboratory, Inc.
365 S. Main St.
PO Box 2081
Memphis, TN 38102
B. Lee Keating
Larry L. Kennon
(A, D, F)

30. Laucks Testing Laboratories, Inc.
1008 Western Ave.
Seattle, WA 98104
James M. Owens
(E)

31. Law & Company
635 Angier St., N.E.
PO Box 1558
Atlanta, GA 30301
John H. Lynch
William W. McBee
(A, B, D, F, I, N, O, P)

32. Mid-Continent Laboratories, Inc.
1354 Madison Ave.
PO Box 1521
Memphis, TN 38101
Donald E. Britton
(A, C, D, F)

33. Morning Star Laboratories, Inc.
4480 Pacific Blvd.
PO Box 58714
Vernon, CA 90058
James A. Laubscher
(D, I, K, N, P)

34. Morris Testing Laboratories
PO Box 803
Macon, GA 31202
John Wieters
(A, B, C, D, N, O)

35. New Jersey Feed Laboratories
910 Pennsylvania Ave.
PO Box 357
Trenton, NJ 08603
Carl W. Schulze
(D, K)

36. Nippon Yuryo Kentei Kyodai
No. 9, 3-chome, kiagandori
Yokohama, Japan
H. Hirayama
(C, I)

37. Northwest Laboratories
1530 First Ave., S.
Seattle, WA 98134
Clyde J. Ambacher
(I)

38. Pattison's Laboratories
211 E. Monroe St.
PO Box 346
Harlingen, TX 78550
Kenneth J. Kalens
Burt O. Pattison
(A, D, G)

39. Pope Testing Laboratories
2618-1/2 Main St.
PO Box 903
Dallas, TX 75221
Leon S. Hunter
R. Cullen Pope
(A, B, D, F, J, K, O)

40. Porter Testing Laboratory
6 S.E. 4th St.
PO Box 25303
Oklahoma City, OK 73125
Deropha J. Porter
(A, B, D, F, I)

41. SGS Control Services, Inc.
2740 Indiana Ave.
PO Box 1328
Kenner, LA 70063
John W. Thomas
(C, D, F, I)

42. SGS Control Services, Inc.
20 Lafayette St.
Carteret, NJ 07008
Michael L. Valletta
Frank W. Weber
(D, F, I)

43. SGS Control Services, Inc.
General Testing Laboratories Division
1001 E. Pender St.
Vancouver, BC Canada V6A 1W2
Walter B. Sizer
(D, I, K, L)

44. Southern Testing and Research
607 Park Ave.
PO Box 350
Wilson, NC 27893
W.A. Bridgers, Sr.
(B, D)

45. Southwell Laboratory
1838 S.W. 13th St.
PO Box 25001
Oklahoma City, OK 73112
Joseph R. Southwell
(A, B, C, D, G, I)

46. Southwestern Laboratories, Inc.
2900 Cullen Ave.
PO Box 1379
Fort Worth, TX 76101
Doak C. Melear, Jr.
(A, B, D, G)

47. Texas Testing Laboratories, Inc.
1610 S. Laredo St.
San Antonio, TX 78207
John G. Caran
(B, E)

48. Thionville Laboratories, Inc.
5440 Pepsi St.
PO Box 23687
New Orleans, LA 70183
Paul C. Thionville
(B, C, D, F, I, K, L, M, N, O, P)

49. Woodson-Tenent Laboratories, Inc.
1805 E. 5th St.
PO Box 5341
North Little Rock, AR 72119
Guy E. Moore
Thomas J. Moore
(A, C, D, F, I, K)

50. Woodson-Tenent Laboratories, Inc.
Atlas Circle
PO Box 1097
Gainesville, GA 30501
Lela I. Vines
(B, C, D, K)

51. Woodson-Tenent Laboratories, Inc.
3507 Delaware Ave.
PO Box 1292
Des Moines, IA 50305
Ardin L. Backous
John R. Ledin
William G. Slagle
(C, D, H, I, K)

52. Woodson-Tenent Laboratories, Inc.
PO Box 845
Clarksdale, MS 38614
James E. Williams
(A, C, D, G)

53. Woodson-Tenent Laboratories, Inc.
South Church St.
PO Box 604
Goldston, NC 27252
H. Newton Beavers
(C, D, I, L)

54. Woodson-Tenent Laboratories, Inc.
345 Adams St.
PO Box 2135
Memphis, TN 38101
Warren B. Harris
Sandra L. Holloway
Arthur C. McConnell
John F. Peden
Richard Wall
(A, B, C, D, F, I, K, N, O, P)

A. **Cottonseed**
1, 4, 5, 7, 8, 9, 17, 19, 21, 22, 29, 31, 32, 34, 38, 39, 40, 45, 46, 49, 52, 54

B. **Peanuts**
17, 19, 21, 31, 34, 39, 40, 44, 45, 46, 47, 48, 50, 54

C. **Soybeans**
7, 9, 10, 17, 21, 25, 28, 32, 34, 36, 41, 45, 48, 49, 50, 51, 52, 53, 54

D. **Oil Cake and Meal, Protein Concentrates**
3, 4, 6, 7, 8, 9, 10, 11, 13, 15, 16, 17, 19, 20, 21, 24, 25, 26, 28, 29, 31, 32, 33, 34, 35, 38, 39, 40, 41, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52, 53, 54

E. **Oil Cake and Meal without Fiber, Protein Concentrates without Fiber**
1,18, 30, 47

F. **Cottonseed Oil, Soybean Oil and Other Fatty Oils**
2, 7, 9, 10, 13, 14, 15, 17, 19, 21, 29, 31, 32, 39, 40, 41, 42, 48, 49, 54

G. **Cottonseed Oil and Other Cup Refined Fatty Oils**
16, 18, 38, 45, 46, 52

H. **Soybean Oil and Other Column Refined Fatty Oils**
20, 51

M. Fish Solubles
7, 10, 28, 48

N. Aflatoxins in Cottonseed and Cottonseed Products
1, 3, 5, 31, 33, 34, 48, 54

O. Aflatoxins in Corn and Corn Products
24, 31, 34, 39, 48, 54

P. Aflatoxins in Peanut and Peanut Products
31, 33, 48, 54

I. Tallow and Grease
2, 6, 7, 10, 12, 13, 14, 15, 16, 20, 21, 23, 25, 27, 31, 33, 36, 37, 40, 41, 42, 43, 45, 48, 49, 51, 53, 54

J. Cellulose Yield (Linters)
19, 21, 39

K. Fish Meal
7, 21, 28, 33, 35, 39, 43, 48, 49, 50, 51, 54

L. Fish Oil
7, 10, 14, 43, 48, 53

New AOCS staff

Sue Heiser has returned to the AOCS staff in Champaign, replacing Gretchen Baumann as executive secretary to James Lyon, AOCS executive director. Mrs. Heiser had worked part-time at AOCS in the bookkeeping department for approximately three years through late 1977.

Suzi Carmichael also is new to the AOCS. She operates the typesetting equipment that turns manuscripts into galley proofs and camera-ready copy for *JAOCS* and *Lipids.* The former typesetters were Betty Schopperth and Colleen Walker, who, after resigning this spring, gave birth to a boy and a girl, respectively.

Members

President's Club and Honor Roll

The members listed here have qualified for either the AOCS President's Club or President's Honor Roll. All current members who successfully recruit at least one new member qualify for Club membership. Successful recruitment of at least three new members is the qualification for the more prestigious Honor Roll. All Club and Honor Roll members will receive further recognition and the opportunity to participate in other special programs and activities. Forms for use in recruiting new members are available from the AOCS Headquarters.

Seven

David L. Berner

Six

Andrew P. Menasian

Five

Frank C. Naughton

Three

James T. Brenna
Ralph T. Holman

Two

John R. Euber
Edward Hahn
Timothy L. Mounts
Glenn W. Patterson
Jose A. Vasconsellos R.
Teturo Wada
Norman H. Witte

One

Moghisuddin Ahmad
Donald B. Appleby
Taboraga J. Aramayo
Paritosh Bhattacharya
Ronald J. Boczkowski
Michael J. Boyer
Dean K. Bredeson
Donald E. Britton
Leon Cabes, Jr.
Juan Chavez-Peraza
Kim O. Chung
James C. Clouse
Alleyne F. Colebrook
Charles Cyopik
James P. Duffy
Francis Duneczky
Jose Esteves
Deo Folco
Hector Gil A.
Stewart E. Gloyer
Horace P. Gormley, Jr.
Christopher R. Gorski
Reuben H. Grinstein
C. Carol Guilbert
Earl G. Hammond
William A. Hansen
Robert C. Hastert
Lynn A. Hawkins
John E. Heilman
Norman C. Heins
James J. Jasko
Robert G. Jensen
Robert W. Johnson
Lynn A. Jones
Mark Keeney
David R. Kime
C. Louis Kingsbaker
William Link
Erland R. Lowrey
Charles D. McCorkle
Dale M. McDaniel
Mary C. McKenna
Salvatore N. Milazzo
David E. Minnikin
Govind R. Mirmira
Ragnar Ohlson
Stuart Patton
Ananda G. Rao
Edward J. Reid
Herbert L. Rice
John H. Rice
Louis L. Richardson
Paul Schur
Edward S. Seguine
Verendra K. Sharma
B.S.K. Shastry
Hiroyuki Shimasaki
Irwan M. Siregar
Thomas H. Smouse
Andrew A. Spark
Ralph Stern
Paul C. Thionville
John W. Thomas
Nancy D. Turner
Leslie R. Watkins
Penelope A. Wells

The following persons had applied for membership in the American Oil Chemists' Society through mid-July, 1980. If an applicant had been invited to join by an AOCS member, the member's last name appears in parentheses at the end of the paragraph.

Gerald J. Buonopane, graduate student, University of Connecticut, Box U-17, Dept. of Nutritional Sciences, Storrs, CT 06268. (Brenna)

Ricardo O. Carvalho, industrial manager and chemical engineer, Wosgrau Industria de Oleos Vegetais Ltda., Km. 1, Rodovia Ponta Grossa Palmeira, Ponta Grossa, Parana, Brazil 84100.

Rebecca L. Dabora, student, Bowdoin College, 159 Davis Rd., Storrs, CT 06268. (Brenna)

Rudolph E. Deck, technical brand manager, Procter & Gamble, 6071 Center Hill Rd., Cincinnati, OH 45224. (Seguine)

Andres Ellena-England, professor, Facultad de Ingenieria Quimica, Universidad del Litoral, Santiago del Estero 2829, 3000 Santa Fe, Santa Fe 3000.

Michael N. Eskin, food chemistry professor, University of Manitoba, Winnipeg, Manitoba, R3T 2N2 Canada. (Smouse)

Nancy L. Fey, graduate student, University of Connecticut, U-17, Storrs, CT 06268. (Brenna)

Robert D. Goodridge, chemistry lab manager, Foster Farms, 14519 W. Collier Rd., Delhi, CA 95315. (McDaniel)

David F. Horrobin, research director, Primrose Research Inc., Suite 11, 245 Victoria, Montreal, Quebec, Canada H3Z 2M6. (Holman)

Halina Kozlowska, professor, University of Agriculture, 43 Kortowo, Olsztyn, Poland, 10-937.

Norman F. Kruse, senior process engineer, CPC International, Corn Products Unit, 6400 Archer Ave., PO Box 347, Summit-Argo, IL 60501. (Witte and Bredeson)

Osvaldo F. Mercuri, associate professor, Instituto Fisiologia—Facultad Medicina, Calle 60 y 120, La Plata, Argentina 1900. (Berner)

M.T. Pelaez, student, University of the Americas, 14 Av. Rosendo Marquez, Col La Par, Puebla, Puebla, Mexico. (Vasconsellos R.)

Robert H. Pothast, plant superintendent—soybean milling, A.E. Staley Mfg. Co., 2200 East Eldorado St., Decatur, IL 62525. (Witte)

John D. Radcliffe, research nutritionist, Illinois Institute of Technology Research Institute, 10 West 35th St., Chicago, IL 60616. (McKenna)

Denis K. Tompkins, managing director, Cargo Superintendents Co. (A/Sia) Pty. Limited, 74/76 Whiting St., Artarmon, N.S.W., Australia 2064.

Annel M. Visapaa, chemist, Finnish Customs Laboratory,

Members

14 Tekriikantie, 02150, Espoo 15, Finland.

Claire Vogleman, president, Scott Daniel Enterprises Inc., 1 River Road, Edgewater, NJ 07020. (Berner)

Craig E. Yendell, senior engineer, The Chester Engineers, 2002 Hogback Rd., Suite 16, Ann Arbor, MI 48104. (Boyer)

New corporate member

Productos Espanoles Inc., PO Box 117, Manati, Puerto Rico 00701, has joined AOCS as a corporate member. The company was invited to membership by William Link. Paul Schlapfer, quality control manager, will be the firm's representative. □

Calendar

Continued from page 668A.

General Chairman, Sydney W. Fleming, Engineering Physics Laboratory, Experimental Station, E-357, E.I. duPont de Nemours & Co., Wilmington, DE 19898.

International Federation for Automatic Control Workshop on "The Impact of Automatic Control and Information Systems on Organization in the 1980s, Sept. 29-Oct. 1, 1980, Youngstown State University, Youngstown, OH. Contact: A.R. Curran, Social Effects of Automation Committee, c/o Department of Mangement, Youngstown State University, Youngstown, OH 44555 USA.

October

World Conference on Plastics and Rubber Education, sponsored by the Yugoslavian Society of Plastics and Rubber Engineers, Oct. 1-3, 1980, Zagreb, Yugoslavia. Contact: Wolfgang A. Mack, U.S. Coordinator, Werner & Pfleiderer Corporation, 160 Hopper Ave., Waldwick, NJ 07463.

Frontiers in Nutrition (second annual course), Oct. 2-4, 1980, Hilton Head Island, SC, sponsored by the Georgian Institute for Human Nutrition. Medical College of Georgia and American Society for Clinical Nutrition. Contact: E.B. Feldman, Georgia Institute of Human Nutrition, Faculty Pavilion 101, Medical College of Georgia, Augusta, GA 30912 (tele: 404 828-4861).

ASTM Committee E-19 on Chromatography, sponsored by ASTM, Oct. 5-10, 1980, Hyatt Kansas City, Kansas City, MO. Contact: Kitty Riley, ASTM, 1916 Race St., Philadelphia, PA 19103.

International Symposium on Energy and Food Industry, Oct. 6-8, 1980, sponsored by the Commission Internationale des Industries Agricoles et Alimentaires (CIIA) and the International Union of Food Science and Technology (IUFoST), in cooperation with the Spain Ministry of Agriculture, Madrid, Spain. Contact: Direcion General de Industrias Agrarias (Simposio 80). Ministerio de Agricultura, Paseo Infanta Isabel 1-Madrid 7, Spain (information in Spanish) or CIIA, C.P. 470-08, 75366 Paris Cédex 08, France. (Information in language other than Spanish).

"Expochem '80," international exposition of analytical instrumentation in the industrial and biomedical fields, sponsored by the University of Houston, Oct. 6-9, 1980, Astrohall, Houston, TX. Contact: Albert Zlatkis, Chemistry Dept., University of Houston, Houston, TX 77004.

National Renderers Association 47th Annual Convention, Oct. 6-10, 1980, Fairmont Hotel, Denver, CO. Contact: Sandra Yeiser, National Renderers Association, 3150 Des Plaines Ave., Des Plaines, IL 60018.

Symposium on Pesticide Applications, sponsored by American Society for Testing and Materials, Oct. 7, 1980, Philadelphia, PA. Contact: A. David Lindsay or John Wright, FMC Corporation, 100 Niagara St., Middleport, NY or Ed Sawyer, Floridin Co., Berkeley Springs, WV 25411.

"Formulation for and Utilization of Pigment Dispersion Equipment," Oct. 7, 1980, presented by the Manufacturing Committee of the Cleveland Society for Coatings Technology, Cleveland Engineering and Scientific Center, Cleveland, OH. Contact: Girish Dubey, Cambridgy Coatings, Inc., 5461 Dunham Rd., Cleveland, OH 44137 (tele: 216-475-3800).

Seventh International Naval Stores Conference and Association Meeting, sponsored by the Pulp Chemicals Association, Oct. 7 & 8, 1980, Capital Hilton Hotel, Washington, DC. Contact: Pulp Chemicals Association, 60 East 42nd St., New York, NY 10017.

"Fifth Symposium on Aquatic Toxicology," co-sponsored by the American Society for Testing and Materials and the American Fisheries Society, Oct. 7-8, 1980, Philadelphia, PA. Contact: J. Gareth Pearson, U.A. Army Medical Bioengineering Research and Development Laboratory, Ft. Detrick, Frederick, MD 21701.

"Extrusion Compounding with Additives and Fillers," regional technical conference (RETEC) sponsored by the Newark Section of the Society of Plastics Engineers, Oct. 7-8, 1980, Sheraton Inn, Newark Airport, NJ. Contact: RETEC Publicity Chairman, M.A. Delia, Delia Associates, PO Box 338, Whitehouse, NJ 08888.

ASTM Committee F-21 on Filtration, Oct. 15-16, 1980, Bienville House, New Orleans, LA. Contact: Janet R. Schroeder, ASTM, 1916 Race St., Philadelphia, PA 19103 (tele: 215-299-5529).

"Engineering '80," conference sponsored by the American Association of Engineering Societies, Oct. 16-17, 1980, Marriott Hotel (West Loop), Houston, TX. Contact: Cathy Ann Valentino, American Association of Engineering Societies, Inc., 345 E. 47th St., New York, NY 10017.

Association of Official Analytical Chemists Annual Meeting, Oct. 19-23, 1980, Marriott Twin Bridges Hotel, Washington, DC. Contact: AOAC, Box 540 Benjamin Franklin Station, Washington, DC 20044.

ASTM Committee E-27 on Hazard Potential of Chemicals, Oct. 20-21, 1980, Dutch Inn, Orlando, FL. Contact: Bill Hulse, ASTM, 1919 Race St., Philadelphia, PA (tele: 215-299-5507).

Symposium: "Sensory Evaluation of Product Performance," Oct. 20-22, 1980, sponsored by the Society of Cosmetic Chemists, Hilton Hotel, Stratford-upon-Avon, England. Contact: M. Callingham, 56 Kingsway, London WC2B 6DX, England.

"ISA/80," 1980 International Exhibit and Conference sponsored by the Instrument Society of America, Oct. 20-23, 1980, Astrohall, Houston, TX. Contact: Instrument Society of America, 67 Alexander Dr., PO Box 12277, Research Triangle Park, NC 27709.

94th Annual Meeting of the Association of Official Analytical Chemists, Oct. 20-23, 1980, Marriott Twin Bridges Hotel, Washington, DC. Contact: K. Fominaya, AOAC, 1111 N. 19th St.—Suite 210, Arlington, VA 22209.

ASTM Committee E-46 on Quality Systems, sponsored by ASTM, Oct. 21-23, 1980, Dutch Inn, Orlando, FL. Contact: Joe J. Palmer, ASTM, 1916 Race St., Philadelphia, PA

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19103.

ASTM Committee E-41 on Laboratory Apparatus, sponsored by ASTM, Oct. 22-23, 1980, Dutch Inn, Orlando, FL. Contact: Jim Dywer, ASTM, 1916 Race St., Philadelphia, PA 19103.

"Protective Coatings for the Maintenance of Bridges and Structures," cosponsored by the University of Missouri—Rolla and the Institute for Bridge Integrity and Safety, Oct. 23, 1980, St. Louis, MO. Contact: Norma R. Fleming, conference coordinator, Arts and Sciences Continuing Education, University of Missouri-Rolla, Rolla, MO 65401.

"Analytical Approaches to Flavor Problems," short course sponsored by the American Association of Cereal Chemists, Oct. 27-29, 1980, Howard Johnson's Motor Lodge, Newark Airport, Newark, NJ. Contact: Ruth Nelson, AACC Headquarters, 3340 Pilot Knob Rd., St. Paul, MN 55121.

"Electron Microscopy of Cereals and Cereal Products," short course sponsored by the American Association of Cereal Chemists, Oct. 29-30, 1980, Earle Brown Center for Continuing Education, University of Minnesota, St. Paul, MN. Contact: Ruth Nelson, AACC Headquarters, 3340 Pilot Knob Rd., St. Paul, MN 55121.

"Three R's for the 80's: Research, Resources, and Regulations" Annual Meeting of the Federation of Societies for Coatings Technology, held jointly with the Paint Industries' Show, Oct. 29-31, 1980, Civic Center, Atlanta, GA. Contact: Hugh Lowrey, Federation of Societies for Coatings Technology, 1315 Walnut St., Suite 830, Philadelphia, PA 19107.

International Week of Engineering, Oct. 19-25, 1980, sponsored by the Mexican Federation of Engineering Societies, Mexico City, Mexico. Contact: Julie Gibouleau, American Association of Engineering Societies, 345 E. 47th St., 3rd Floor, New York, NY 10017.

November

National Lubricating Grease Institute Annual Meeting, Nov. 2-5, 1980, Royal Sonesta, New Orleans, LA. Contact: J. Penrod, NLGI, 4635 Wyandotte St., Kansas City, MO 64112.

"Gum Chemistry and Technology," sponsored by American Association of Cereal Chemists, Nov. 10-12, 1980, Howard Johnson's Motor Lodge, Newark, NJ. Contact: Dotty Ginsburg, American Association of Cereal Chemists, 3340 Pilot Knob Rd., St. Paul, MN 55121.

"Improving the Productivity of Technical Resources," conference cosponsored by the IIT Manufacturing Productivity Center, the American Productivity Center and the Technological Institute of Northwestern University, Nov. 11-12, 1980, IIT Research Institute, 10 W. 35th St., Chicago, IL. Contact: Paul Norton, IIT Research Institute.

VII Simposio Nacional and the IV Latinoamericano de Oleaginosos, Nov. 17-19, 1980, Bolso de Cereales Headquarters, Buenos Aires, Argentina. Contact: Oficina de Coordinacion, Corrientes 127, 2° Piso, Capital, Argentina.

"Outlook '81," 57th Annual Agricultural Outlook Conference, sponsored by the U.S. Department of Agriculture, Nov. 17-20, 1980, Washington, DC. Contact: Outlook '81, Rm. 3510-S, USDA, Washington, DC 20250.

International Symposium on "Leukotriene and Lypoxygenases," sponsored by Fondazione Giovanni Lorenzini, Nov. 20-22, 1980, Milan, Italy. Contact: Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23-20121, Milano, Italy.

Lipochemistry Technical and Industrial Symposium, sponsored by the Institut des Corps Gras (ITERG), Nov. 27-28, 1980, Bordeaux, France. Contact: A. Uzzan, Institut des Corps Gras, Etudes et Recherches Techniques, 10/A rue de la Paix, 75002 Paris, France.

December

"Introduction to Food Law," sponsored by the University of Minnesota Office of Special Programs, directed by Prof. T.P. Labuza, Dec. 8-9, 1980, Earle Brown Continuing Education Center, University of Minnesota, St. Paul, MN 55108. Contact: Julie Fountain, Office of Special Programs, 405 Coffey Hall, University of Minnesota, 1420 Eckles Ave., St. Paul, MN 55108.

First Colloquium on Lipid Metabolism and Its Pathology, Dec. 8-11, 1980, sponsored by Universidade Nova de Lisboa, Faculdade de Ciencias Medicas, Departamento de Bioquimica. Contact: Prof. Manuel Judice Halpern, Departamento de Bioquimica, Faculdade de Ciencias Medicas da UNL, Campo dos Martires da Patria, 1100 Lisboa-Portugal.

Society of Cosmetic Chemists Annual Scientific Meeting, Dec. 11-12, New York City. Contact: Program Co-chairmen, Robert L. Goldemberg or Harvey S. Schnur, c/o Society of Cosmetic Chemists, 1995 Broadway, Suite 1701, New York, NY 10023.

1981

"Korchem," chemical engineering exhibition organized by Industrial and Trade Fairs International, Ltd., Feb. 2-9, 1981, Korean Exhibition Center, Seoul, Korea. Contact: Gerald G. Kallman, Kallman Associates, U.S. Representative-Korchem, 30 Journal Square, Jersey City, NJ 07306.

"International Conference on High Temperature Corrosion," sponsored by the National Association of Corrosion Engineers, March 2-5, 1981, Radisson Scottsdale Resort and Racquet Club, Scottsdale (Phoenix), AZ. Contact: Robert A. Rapp, Dept. of Metallurgical Engineering, Ohio State University, 116 W. 19th Ave., Columbus, OH 43210.

Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy, March 9-13, 1981, Atlantic City, NJ. Contact: Richard Obrycki, Exposition Chairman, Pittsburgh Conference, 437 Donald Road, Pittsburgh, PA 15235.

Symposium on Color and Appearance Instrumentation, sponsored by the Federation of Societies for Coatings Technology, Mar. 24-26, 1981, Executive West, Louisville, KY. Contact: Federation of Societies for Coatings Technology, 1315 Walnut St., Suite 832, Philadelphia, PA 19107.

American Chemical Society 181st National Meeting, March 29-April 3, 1981, Atlanta, GA. Contact: American Chemical Society, 1155 16th St. NW, Washington, DC 20036.

"Corrosion/81," sponsored by the National Association of Corrosion Engineers, April 6-10, 1981, Sheraton-Centre and Hotel Toronto, Toronto, Ontario, Canada. Contact: Conference Coordinator, NACE, PO Box 218340, Houston, TX 77218.

"Food Microstructure," program scheduled during Scanning Electron Microscopy/1981 Meeting, April 14-18, 1981, Fairmont Hotel, Dallas, TX. Contact: Om Johari, PO Box 66507, AMF O'Hare, IL 60666.

"First Asian and Pacific Chemistry Congress," co-sponsored by the Singapore National Institute of Chemistry and the Singapore National Academy of Science, April 26-May 1, 1981, Singapore, Contact: Singapore Tourist Promotion Board, 342 Madison Ave., Suite 1008, New York, NY 10017.

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Technical News Feature

The Removal of Organic Substances from Water with Nonvolatile Edible Solvents

R.J. JANDACEK and **R.L. BOHNE**, Procter & Gamble, Miami Valley Laboratories, PO Box 39175, Cincinnati, OH 45247

ABSTRACT

The use of nonvolatile, edible triglycerides for the removal of trace organic materials from water was investigated. All organic substances that were studied were readily removed from water by contact with triglycerides. Solvent extraction with liquid soybean oil reduced the concentrations of benzene and chloroform in water. A fixed bed filter of liquid soybean oil, hydrogenated palm oil and sand removed toluene and chloroform from water. Toluene was removed from water by adsorption with hydrogenated palm oil, a solid fat.

INTRODUCTION

The generally accepted method for the removal of trihalomethanes and other organic materials from water has been adsorption on the surface of activated carbon. Although activated carbon is satisfactory in many water purification situations, a reduction in the cost of the carbon and the expense of its surface regeneration would be desirable.

Moreover, it is possible that other processes may provide advantages in cost and effectiveness compared to carbon adsorption. An alternative to carbon adsorption could be extraction of water with an organic solvent. Solvents can effectively remove organic compounds from water, but trace amounts of the solvent may be distributed into the water. In addition, the volatility of most organic solvents limits their practicality. Solvent extraction might be feasible, however, with nonvolatile, nontoxic solvents that are insoluble in water. We report here a preliminary demonstration that triglyceride fats, nonvolatile foodstuffs, can perform as organic solvents or adsorbents and effectively remove organic compounds from water.

MATERIALS AND METHODS

The water used in all systems was from the deionized water tap at our laboratories. The partially hydrogenated soybean oil (liquid, iodine value 107), and completely hydrogenated palm oil (solid, iodine value 8) were bleached, deodorized, edible fats obtained from the Food Division of Procter & Gamble, Cincinnati, OH. The fatty acid composition of these materials is given in Table I. The Ottawa standard sand and reagent grade benzene, toluene and chloroform were obtained from MC/B, Norwood, OH.

Aqueous solutions were prepared by delivering the lipophile by micropipette to a known volume of water. Vigorous mixing provided dispersion and dissolution, and stepwise dilution gave the desired concentrations.

Two types of assays were used for water analysis. Benzene and toluene in water were analyzed by measurement of the ultraviolet (UV) absorbance of the aqueous phase (249 nm, benzene, and 260 nm, toluene) and comparison with absorbances of standard solutions. Chloroform was analyzed by gas chromatography (GC) by the James M. Montgomery Consulting Engineers, Pasadena, CA.

Three systems were used to demonstrate the reduction of organic material in water with triglycerides. The first of these used a liquid oil in a solvent extraction procedure. The second used an immobilized liquid oil in a fixed bed filter. The third system consisted of a fixed bed filter of a solid fat to remove the organic material by surface adsorption. All systems were at ambient temperature, 22-24 C.

System 1: Soybean Oil Layer on Stirred Water

A solution of the organic material in water (750-875 ml) was poured into each of 2 jars containing magnetic stirring bars. A layer (70-100 ml) of liquid soybean oil was gently poured onto the surface of water in one of the jars. After both jars were closed the water was stirred slowly with teflon-covered magnetic bars. The stirring rate was adjusted so that no visible oil droplets were allowed to form in the water. The area of the oil/water interface was ca. 90 cm^2. At intervals from 0-24 hr, aliquots were taken either by aspiration of the aqueous phase beneath the oil or by pipette from the jar containing only the aqueous solution of the lipophile.

System 2: Fixed Bed Filter

This system was a fixed bed of sand, solid hydrogenated palm oil and liquid soybean oil (5:5:1, v/v) that had been mixed with a spatula. About 100 ml of this mixture was placed in a separatory funnel and 20 ml of the aqueous solution (of lipophile) was poured through it and collected for analysis.

System 3: Fixed Bed, Solid Adsorbent

This system was a fixed bed filter similar to system 2. It consisted of 10 g of a single component, completely hydrogenated soybean oil, in a 125-ml separatory funnel. The aqueous solution (70 ppm toluene) was allowed to pass through the bed of granules of fat. These granules were irregular particles of dimensions of ca. 1 mm x 1 mm x 0.5 mm.

TABLE I

Composition of Fatty Acid Methyl Esters from Soybean Oil, Hydrogenated Palm Oil and Hydrogenated Soybean Oil (Weight %)

	Liquid soybean oil	Hydrogenated palm oil	Hydrogenated soybean oil
C14:0	0.3	1.0	Trace
C16:0	11.6	45.6	10.7
C16:1	--	--	0.2
C18:0	4.8	50.8	84.5
C18:1	47.1	0.6	4.3
C18:2	35.0	--	Trace

RESULTS AND DISCUSSION

System 1: Soybean Oil Layer on Stirred Water

Table II gives the results obtained for the removal of benzene and chloroform from water by this system. About 69% of the benzene and 77% of the chloroform were removed by this extraction process.

System 2: Fixed Bed Filter

Table III shows the change in concentration of toluene in water in 2 different solutions after a single pass through the sand-fat mixture. The removal of toluene was evident at both concentrations. The results for multiple passes of a chloroform solution are also given. Five passes through the filter removed nearly 80% of the chloroform. The decrease in the rate of removal with each pass reflects the reduction of chloroform in the aqueous phase as well as its uptake by the fat.

Since the systems with liquid soybean oil are solvent extraction processes, the "sink" for toxic lipophiles provided by dissolution in the oil is potentially larger than that provided by surface adsorption on activated carbon. Thus the extraction of organic materials by a triglyceride may surpass the 50% reduction that is sought with treatment by activated carbon (1).

System 3: Fixed Bed, Solid Adsorbent

Table IV shows the reduction of toluene in water caused by a single pass through a fixed bed filter of completely hydrogenated soybean oil. Almost 80% of the toluene was adsorbed by this high-melting fat. The reduction of toluene in water by the solid fat, completely hydrogenated soybean oil, suggests that such fats may provide a nontoxic adsorbent that could easily be separated from water.

The purpose of this study was to determine whether or not a triglyceride could remove lipophilic materials from water with systems that maintain a separation of the oil and water phases. All 3 organic substances examined were readily taken up from dilute aqueous solution by the oil phase. This water treatment concept is unique in that it employs the use of organic solvents or adsorbents that are edible and thus nontoxic. Paraffin and mineral oils may also meet this criterion of low toxicity.

The application of this concept to large-scale water treatment may be feasible. Clearly there remain unanswered questions about areas such as fat stability, microbial growth and cost analysis. In addition, the potential transfer of unchanged or hydrolyzed triglyceride to water might occur. The cost of regeneration of triglycerides by a steam deodorization process would need to be determined and compared to that of the reactivation of activated carbon in a furnace. The optimization of mixing and of separation of phases has been only briefly addressed in this study. The data presented here, however, suggest the potential use of nontoxic fats and oils in the removal of toxic lipophiles in water purification.

TABLE II

The Removal of Benzene or Chloroform from Water by Solvent Extraction with Liquid Soybean Oil

Time (hr)	Benzene concentration in water (ppm) Without soybean oil	With soybean oil
0	64	64
1	64	50
2	64	40
18	64	28
21.5	62	< 20

Time	$CHCl_3$ concentration in water (ppb) Without soybean oil	With soybean oil
24 hr	556	126

TABLE III

The Removal of Toluene or Chloroform from Water by a Fixed Bed Filter of Liquid Soybean Oil, Hydrogenated Palm Oil and Sand

	Toluene concentration in water (ppm)	
	Before filtration	After single filtration
(Solution 1)	46	18
(Solution 2)	10	1

Number of passes through filter	Chloroform concentration in water (ppb)
0	949
1	454
3	276
5	200

TABLE IV

The Removal of Toluene from Water with a Solid Fat, Completely Hydrogenated Soybean Oil

Toluene concentration in water (ppm) Before filtration	After single filtration
70	15

REFERENCE

1. Preliminary Assessment of Suspected Carcinogens in Drinking Water, Report to Congress, USEPA, Washington, DC, 1974, p. 47-51.

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Technical

A Water Slurry Method of Extracting Aflatoxin from Peanuts[1]

T.B. WHITAKER and **J.W. DICKENS**, USDA, SEA, AR, North Carolina State University, and **R.J. MONROE**, Department of Statistics, North Carolina State University, Raleigh, NC 27650

ABSTRACT

A water slurry method in which 1100 g of comminuted peanuts was blended with 1500 ml of tap water for 3 min in a blender and the aflatoxin in a 130-g portion of the water slurry was extracted by solvent according to methods similar to those used in Method II of AOAC was compared to the method presently used by the Food Safety and Quality Service, USDA. The proposed water slurry method requires only 180 and 60 ml per sample, respectively, of methanol and hexane compared to the 1650 and 1000 ml, respectively, required by the FSQS method. Blending comminuted peanuts with water reduced the average particle size and distributed the contaminated particles throughout the slurry. Ninety-four percent of the blended particles passed a sieve with 149-μ openings compared to only 66% of the unblended product. Variance among analyses with the FSQS method did not differ significantly from the variance among analyses with the slurry method. However, analyses with the slurry method averaged 16% more aflatoxin than with the FSQS method.

INTRODUCTION

The aflatoxin assay procedure required by the Peanut Administrative Committee for all commercial lots of shelled peanuts produced in the United States is a modification of AOAC Method II by the Food Safety and Quality Service (FSQS) of the U.S. Department of Agriculture (1-3). The FSQS procedure requires solvent-extraction of a 1100-g subsample of comminuted peanut kernels with 1650 ml of methanol, 1350 ml of water, 1000 ml of hexane and 22 g of sodium chloride. In addition to being costly, the solvents are an important energy resource and the used solvents are difficult to dispose of without environmental pollution.

The subsampling error in aflatoxin tests on peanuts is inversely proportional to the weight of the subsample of comminuted peanuts from which the aflatoxin is extracted (4). The ratio of solvents to comminuted peanuts is fixed by requirements for complete aflatoxin extraction and for subsequent analytical procedures. The amount of comminuted peanuts extracted can be reduced without increasing subsampling error by reducing the particle size. Particle size cannot be further reduced with the subsampling mill used presently (5).

A subsample of well-blended peanut paste made from a sample of comminuted peanuts may be used for extraction, but the cost of equipment and the time required for preparing the paste and cleaning the equipment presents problems. Extraction of a subsample of a slurry made by blending a sample of comminuted peanuts in water is a feasible alternative. Blenders presently available in aflatoxin laboratories may be used, cleanup is easy and the blending process reduces the particle size. Use of water slurries in aflatoxin tests has been studied previously (6).

The proposed slurry method consists of extracting aflatoxin with solvent from a 130-g sample of a slurrry formed by blending 1100 g of comminuted peanut kernels, 1500 ml of water and 22 g of sodium chloride in a Waring Blender. In the FSQS method used presently, aflatoxin is extracted from the entire 1100-g subsample. The aflatoxin is probably uniformly distributed throughout the 3000 ml of methanol/water solution; there probably is no sampling error when only 50 ml of the solution is assayed for aflatoxin. However, in the proposed slurry method, aflatoxin is only partially extracted from the peanuts by the water (6) and on the average only 54.5 g of peanut material is in each 130-g sample of slurry. Thus replicated 130-g samples from the same slurry may contain different quantities of aflatoxin. The magnitude of variation in aflatoxin content of the 130-g slurry samples is affected by the size of peanut particles in the slurry (4) and by the differences in total particle weight among equal-weight samples of the slurry.

In this study we measured the size of peanut particles in the slurry and the variation in total particle weight among replicated 130-g samples of the slurry, optimized a procedure for extracting aflatoxin from the slurry, and compared the variance among analyses obtained by the slurry method and by the FSQS method used presently.

PROCEDURE

The proposed slurry method and the FSQS method are outlined in Table I. In step 1 of the slurry method, the ratio of peanut material to water is such that the slurry is fluid enough to blend properly but thick enough to prevent the larger particles in the slurry from settling out of suspension when the slurry is left standing for 5 min. One of the experiments described later determined that the 180 ml of methanol used in step 2 adequately extracts the aflatoxin from 130 g of slurry. The addition of 25 ml of a 10% solution of sodium chloride in step 4 reduces the cloudiness of the chloroform in step 5.

Particle Size Distribution

A slurry was prepared by the procedure described in Table I. All of the 2622 g of slurry was then washed through U.S. Standard Sieves (#8, #16, #30, #50 and #100) with a light spray of tap water. The dry weight of material retained on each screen was determined after the material was dried in a forced draft oven at 100 C for 24 hr. For determination of the size distribution before blending into a slurry, a 1100-g sample of comminuted peanuts was also washed through the sieves and treated in a similar manner.

Variability in Weight of Dried Slurry Samples

A slurry was prepared according to the procedure described in Table I. After blending, the slurry was allowed to stand for 5 min in the blender cup. Then samples of slurry were obtained by pouring 130 g of slurry into each of 19 6-inch aluminum pie pans numbered 1 through 19 in the order

[1]Paper No. 6189 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC.

TABLE I

Comparison of the Slurry Method and the FSQS Method

SLURRY METHOD

1. Blend 1100 g of peanuts comminuted in a subsampling mill (5) with 1500 ml of tap water and 22 g of sodium chloride for 3 min at medium speed in a 1-gal blender cup.
2. Blend 130-g of slurry from step 1 with 180 ml of methanol and 60 ml of hexane for 30 sec at high speed in a 1-qt blender.
3. Centrifuge material from step 2 in a 500-ml centrifuge bottle at a rotational centrifugal force of 2500 G for 20 min.
4. Transfer 50 ml of the methanol/water/aflatoxin solution to a 125-ml separatory funnel, add 25 ml of 10% sodium chloride solution, and blend the contents by vigorously shaking the stoppered funnel for 30 sec.
5. Add 50 ml of chloroform to the separatory funnel, shake the stoppered funnel for 30-60 sec, let the fractions separate, and drain the chloroform into a beaker.
6. Complete the assay according to AOAC Method II (2).

FSQS METHOD

1. Blend 1100 g of peanuts comminuted in a subsampling mill (5) with 1650 ml of methanol, 1350 ml of water, 1000 ml of hexane, and 22 g of sodium chloride for 2 min at high speed in a 1-gal blender cup.
2. Centrifuge ca. 500 ml of material from step 1 in a 500-ml centrifuge bottle at a rotational centrifugal force of 2500 G for 20 min.
3. Transfer 50 ml of the methanol/water/aflatoxin solution to a 125-ml separatory funnel and add 50 ml of chloroform.
4. Stopper and shake the separatory funnel for 30-60 sec, let the fraction separate and drain the chloroform into a beaker.
5. Complete the assay according to AOAC Method II (2).

that the samples were poured from the blender. The weights of the samples (comminuted peanuts + salt) were determined after they were oven-dried at ca. 54 C for 72 hr and then at ca. 100 C for 24 hr. This experiment was repeated 3 times. The moisture content of the comminuted peanuts used in this study was determined to be 6.0% wet basis by drying 1200 g in the same manner as the slurry samples. The oven-dried material from each sample of slurry was assumed to consist of peanuts and 1.09 g of sodium chloride. The oven-dry weight of peanuts in each slurry sample was adjusted to reflect the original 6.0% wet-basis moisture content of the comminuted peanuts used to make the slurry.

Optimization of Aflatoxin Extraction

In this experiment, we determined the effect of methanol concentration on aflatoxin extraction from samples of slurry prepared by the procedure already described. Four 130-g samples of the slurry were blended with 90, 135, 180, or 225 ml of methanol and with 60 ml of hexane in a 1-quart blender for 0.5 min. A 50-ml portion of the methanol/water extract was assayed for aflatoxin according to the slurry procedure already described. Quantification of aflatoxin was made for all 4 samples on the same TLC plate. This procedure was repeated until 10 samples of slurry were assayed using each quantity of methanol.

Comparison of the Variance among Assays Made with the FSQS Method and with the Slurry Method

This experiment compared the variability of FSQS aflatoxin assays with the variability of assays made with the slurry method. In each case the variance reflects the expected error when a 1100-g sample of comminuted peanuts is analyzed by the respective analytical method. Two estimates of the variability associated with each method were made in this study. In test 1, ca. 44 kg of aflatoxin-contaminated peanuts were comminuted in a subsampling mill. The product was riffle-divided into 40 1100-g samples which were analyzed in pairs: one by the FSQS method and one by the slurry method. The extract from each sample in the pair was spotted 6 times onto the same TLC plate. From the 120 estimates of total aflatoxin per method (6 replications x 20 samples), the variance associated with each method was computed by the analysis of variance.

For test 2, ca. 71 kg of comminuted material was riffle-divided into 64 1100-g samples. Thirty-two pairs of samples were assayed in the manner described for test 1 except that the extract from each sample was spotted 3 times onto the same plate. From the 96 estimates of total aflatoxin per method (3 replications x 32 samples), the variance associated with each method was computed by the analysis of variance.

Comparison of Averaged Assays by the FSQS Method and the Slurry Method

For comparison of the averaged assays by the 2 methods to be meaningful, the average aflatoxin concentration in the samples assayed by each method must be the same. To insure that the averaged aflatoxin concentrations of the samples assayed by each method were the same, large quantities of well-blended and riffle-divided comminuted peanuts were used in each of 4 separate tests. Tests 1 and 2, described in the previous section, along with 2 additional tests (3 and 4) were made to compare the averaged aflatoxin assays of the 2 methods.

In test 3, 64 1100-g samples were prepared in the same way as in test 2. Each of 32 samples were blended in methanol/water/hexane according to the FSQS procedure. Three 250-ml portions of the blended material from each sample were centrifuged and 50-ml quantities of methanol/water from each of all 96 portions were combined. Each of the remaining 32 samples was made into a slurry. Three 130-g portions of slurry from each sample were extracted and centrifuged according to the slurry procedure, and 50 ml of methanol/water from each of all 96 portions were combined.

Twenty 50-ml portions of the methanol/water from the FSQS treatment and twenty 50-ml portions of the methanol/water from the slurry treatment were then assayed for aflatoxin according to the respective procedure. Two 50-ml portions (one from each treatment) were assayed at the same time, and 3 spots of extract from each of the 2 50-ml portions were placed onto the same TLC plates. Totals of 60 estimates of the aflatoxin concentration (20 TLC plates x 3 estimates/plate) in the methanol/water extract from each procedure were thus obtained.

Test 4 was conducted on peanut paste made from aflatoxin-contaminated peanuts with a laboratory peanut-butter mill. The thoroughly blended paste was divided into 8 1100-g samples, and 4 of the samples were each made into a slurry. Ten 130-g portions of slurry from each sample were extracted and centrifuged according to the slurry procedure. Then 50 ml of methanol/water from each of all 40 portions were combined. The remaining 4 samples were carried through step 1 of the FSQS method. Ten 250-ml portions of the blend from each sample were centrifuged according to the FSQS procedure. Then 50 ml of methanol/water from each of all 40 protions were combined.

Twenty-six 50-ml portions of methanol/water from the FSQS treatment and 26 50-ml portions from the slurry treatment were then assayed for aflatoxin according to their respective procedures. Two 50-ml portions (one from each treatment) were assayed at the same time, and 3 spots of extract from each of the 2 50-ml portions were placed onto the same TLC plate. A total of 104 estimates of the aflatoxin concentration (26 TLC plates x 4 estimates/plate) in the methanol/water extract from each procedure was thus obtained.

RESULTS

Proposed Slurry Method

A 1-quart blender is the only additional piece of equipment needed to change from the FSQS procedure to the slurry procedure, because most aflatoxin laboratories have balances suitable for weighing 130- g samples of slurry. The only additional time needed for the slurry procedure is the time required for step 2. The 130 g of slurry contains 54.5 g of peanuts. Only 10.7 g of peanuts is represented in the 50 ml of chloroform extract compared to 18.3 g for the FSQS method. If both methods have the same lower limit for detection of a quantity of extracted aflatoxin, the lowest detectable concentration in peanuts analyzed by the slurry method would be 1.7, i.e., 18.3/10.7 times higher than the lowest detectable concentration in peanuts analyzed by the FSQS method.

Particle Size Distribution

Considerable comminution occurs during preparation of the slurry (Table II). Ninety-four percent of the peanut particles passed through a #100 sieve after blending whereas only 66% passed before blending. As previously mentioned, subsampling error is reduced by comminuting more finely. Comminution is an important aspect of the slurry method because it helps make the 54.5 g of peanut material in the 130-g sample of slurry more representative of the 1100-g sample used to make the slurry.

TABLE II

Percentages of Peanut Material that Passed the Designated Sieves before Blending into a Slurry and after Blending

U.S. standard sieve number	Sieve opening (microns)	Percent material passing sieve before and after blending: Before	After
8	2380	100	100
16	1190	98	100
30	590	86	100
50	297	70	97
100	149	66	94

TABLE III

Distribution of the Weights (in g) of Peanut Material among 130-g Samples of Water Slurry

Sample number	Replication 1	2	3	Avg
1	54.80	55.20	54.32	54.77
2	54.80	54.30	54.43	54.51
3	54.80	54.46	54.32	54.53
4	55.66	54.34	54.53	54.84
5	54.98	54.46	54.53	54.66
6	54.47	54.46	54.70	54.54
7	53.36	54.46	54.53	54.12
8	53.84	54.40	54.53	54.26
9	55.06	54.40	54.43	54.63
10	54.26	54.51	54.32	54.36
11	54.16	53.63	54.43	54.07
12	54.16	54.35	54.16	54.22
13	54.32	55.10	54.70	54.71
14	54.16	54.46	54.32	54.31
15	53.99	54.34	54.59	54.31
16	55.23	54.24	54.64	54.70
17	54.37	54.35	54.64	54.45
18	54.37	55.10	54.75	54.74
19	54.70	54.89	54.64	54.74
Avg	54.50	54.50	54.50	54.50
SD	0.54	0.36	0.16	0.23
CV(%)	0.99	0.66	0.29	0.43

Variability of Peanut Weight in Slurry Samples

Data from the test on the weight of peanuts in 19 samples of slurry (Table III) indicate that the weights are in good agreement among the samples of slurry. This agreement is important because the calculations of aflatoxin concentrations for the slurry method are based on 54.5 g of peanuts in the sample of slurry. Although there apparently is a slight correlation between sample number and the weight of peanuts in the sample, the differences in the weight of samples poured from the top of the container (low sample numbers) and the weight of samples poured from the bottom of the container (high sample numbers) do not indicate a significant amount of settling during the 5 min the slurry was left standing.

Optimization of Aflatoxin Extraction

The average concentration (ppb) of aflatoxin determined for samples of peanuts when different amounts of methanol were used in step 2 of the slurry method are shown in Table IV. At the 95% level of confidence, significantly less aflatoxin was extracted with 90 ml than with 135, 180 or 225 ml of methanol. Statistically significant differences were not found among the amounts of aflatoxin extracted by the 3 larger quantities of methanol. Based on this test, 180 ml, rather than 135 ml, of methanol was designated in step 2 of the slurry method to give some margin of safety. The effect of blending time in step 2 was also studied. Because this experiment showed no difference in assays between 0.5 and 3.0 min of blending time, the 0.5 min blending time was specified by the authors.

Comparison of the Variance among Assays with the FSQS Method and with the Slurry Method

The results of an analysis of variance on the 120 assays by

TABLE IV

Averages of 10 Determinations of Aflatoxin Concentration in Samples of Peanuts when Different Quantities of Methanol Were Used in Step 2 of the Slurry Method

	Quantity of methanol (ml): 90	135	180	225
Methanol:water ratio	55:45	65:35	71:29	75:25
Aflatoxin concentration (ppb)	28.2[a]	43.7[b]	42.0[b]	49.5[b]

$LSD_{.05}$ = 10.97 ppb.

[a,b]Concentrations with no letter in common are significantly different at 5% level.

TABLE V

Results of Analysis of Variance and Related Statistical Data for Tests 1 and 2

Source	Test 1: FSQS	Test 1: Slurry	Test 2: FSQS	Test 2: Slurry
Variance	1763	2240	182	252
Degrees of freedom	107	107	94	94
Standard deviation (PPB)	40	46	12	15
Mean assay (PPB)	159	179	58	66
Coefficient of variation	26	26	23	24

Null Hypothesis: Slurry variance = FSQS variance.
F test for test 1: F = 2240/1763 = 1.27; P = 0.110.
F test for test 2: F = 252/182 = 1.38; P = 0.060.

TABLE VI

Comparison of Assays with the FSQS Method and with the Slurry Method

Test number	Assay method	Aflatoxin (ppb)				
		B1	B2	G1	G2	Total
1	FSQS	72.6	16.1	56.6	13.3	158.6
	Slurry	87.7	17.5	57.9	15.5	178.6
	% Diff[a]	20.8	8.7	2.3	16.5	12.6
2	FSQS	36.1	5.8	12.8	3.0	57.7
	Slurry	43.7	6.2	13.4	3.2	66.5
	% Diff	21.1	6.9	4.7	6.7	15.3
3	FSQS	40.3	6.3	16.0	4.9	67.5
	Slurry	44.4	6.8	18.1	6.4	75.3
	% Diff	10.2	7.9	13.1	30.6	11.6
4	FSQS	17.9	3.8	10.3	2.7	34.7
	Slurry	21.2	4.6	12.7	4.3	42.8
	% Diff	18.4	21.1	23.3	59.3	23.3
Avg	FSQS	41.7	8.0	23.9	6.0	79.6
	Slurry	49.3	8.8	25.5	7.4	90.8
	% Diff	17.6	11.2	10.9	28.3	15.7

[a]% Diff = 100 (slurry-FSQS)/FSQS.

the slurry method and by the FSQS method in test 1 and on the 96 assays by each method in test 2 are given in Table V. The null hypotheses, that the variance among the slurry assays and the variance among the FSQS assays are equal, cannot be rejected by the F test for test 1 ($F = 1.27$) or for test 2 ($F = 1.38$). Failure of the experiment to show a statistically significant difference between variances for the 2 methods indicates that the error introduced by using the 130-g sample of slurry (54.5 g of peanuts) is negligible.

Comparison of Averaged Assays by the FSQS Method and the Slurry Method

For all tests the average aflatoxin values were higher for the slurry method than for the FSQS method (Table VI). The analysis of variance showed that all of these differences were significant at the 95% confidence level. Since a large number of 1100-g samples that were riffle-divided from comminuted peanuts were used in the tests, the true average aflatoxin concentration in the samples assayed by the 2 methods probably was about the same.

According to previous variance studies (4) the average aflatoxin concentration in the samples analyzed by the FSQS and slurry methods were predicted not to differ by more than 10 ppb in test 1, 4.8 ppb in test 2 and 5.6 ppb in test 3, 95% of the time. The difference in the average aflatoxin concentrations of the 4 1100-g samples of peanut paste used for each method in test 4 probably was also very small because of the homogeneity of the paste. In addition, there is only a 6% chance that the aflatoxin concentration in the samples chosen for assay by the slurry method in all 4 tests would be higher than in the samples chosen for assay by the FSQS method. Further studies are needed to determine the cause of the differences in the averages of the assays by the 2 methods.

REFERENCES

1. Dickens, J.W., JAOCS 54:225A (1977).
2. AOAC: Natural Poisons, Chapter 26 from Official Methods of Analysis. 12th Edition, Association of Official Analytical Chemists, Washington, DC, 1975, p. 7,16.020
3. United States Department of Agriculture Inspector's Instructions for Aflatoxin, USDA Food Safety and Quality Service, Washington, DC 1975.
4. Whitaker, T.B., J.W. Dickens and R.J. Monroe, JAOCS 51: 214 (1974).
5. Dickens, J.W., and J.B. Satterwhite, Food Technol. 23:950 (1969).
6. Velasco, J., and S.L. Morris. J. Agric. Food Chem. 24:86 (1976).

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Partial Argentation Resin Chromatography (PARC): I. Effect of Percent Silver on Elution and Separation of Methyl Octadecadienoate Isomers

R.O. ADLOF, H. RAKOFF and **E.A. EMKEN,** Northern Regional Research Center, Agricultural Research, Science and Education Administration, USDA, Peoria, IL 61604

ABSTRACT

Partial argentation resin chromatography (PARC) for the separation of octadecadienoate ester isomers was investigated. In comparison to saturated silver resin chromatography, the time necessary to elute methyl *cis,cis*-octadecadienaotes was dramatically shortened when columns containing sulfonic acid ion exchange resin silvered in the range of 60-90% of theoretical (meaning 60-90% of the sulfonic acid protons in the resin were replaced by silver ions) were used. Methods for preparation and silvering of the resin are discussed. The XN1010 resin (Rohm and Haas) was analyzed for total sulfonic acid groups and the amount of silver that can be incorporated by one or 2 treatments with silver nitrate was determined. A series of partially silvered resin columns was prepared and samples of methyl linoleate were eluted to study the effect of the percentage silvering on elution volumes and peak shapes. Twenty-gram samples of mixtures of *cis,trans-* and *trans,trans-* and of *trans,cis-* and *cis,cis*-methyl 12,15-octadecadienoates were separated on a 91% PARC (91% silvered) column.

INTRODUCTION

Separation of geometric isomers of unsaturated fatty methyl esters using counter-current distribution, silver nitrate impregnated silica gel, thin layer chromatography (TLC) and silver resin chromatography has been reviewed recently by Scholfield (1).

The separation of monoenoic and dienoic fatty ester isomers using silver saturated, macroreticular cation exchange resins (silver resin chromatography) has been reported (2-7). Although the separation of 10- to 20-g samples of monoenoic ester isomers by this technique was easily accomplished, the *cis,cis*-dienoic and *cis,cis,cis*-trienoic esters required long elution times and large volumes of methanol and resulted in poor peak shapes. These all-*cis* polyunsaturated fatty esters could be displaced from the silver resin column and their elution times thus shortened by the addition of 1-hexene to the eluting solvent (6).

This paper reports the effect of partial silvering of the XN1010 ion exchange resin (where only a portion of the total sulfonic acid protons is replaced by silver ions) on the retention times of methyl linoleate and the application of partial argentation resin chromatography (PARC) for separation of mixtures of *trans,trans-* and *cis,trans-* and of *trans,cis-* and *cis,cis*-octadecadienoate esters. This technique of replacing only a portion of the sulfonic acid protons on a cationic exchange resin with silver ions will be referred to as partial argentation resin chromatography (PARC).

EXPERIMENTAL PROCEDURES

Materials

Amberlyst XN1010 ion exchange resin is a sulfonated, cross-linked, polystyrene-type resin, which is supplied in the hydrogen form. It is hard, dry, spherical and gray-black in appearance. The resin (16- to 50-mesh particles [U.S. Standard Sieve]) has a skeletal density of 1.35 g/ml, a bulk density of 5.45 kg/m^3 and a surface area of 500 sq/g. The mean pore diameter is 50 Å units and the resin has a cation exchange capacity of 3.1 milliequivalents (meq)/g (8).

Methyl linoleate was separated by PARC from trans-esterified safflower oil (PVO International, San Francisco, CA). The isomeric methyl 12,15-octadecadienoates-9,10-d_2 were synthesized as previously reported (9).

Methods

Chromatographic separations, unless specifically noted, were made through an 85 × 0.6-cm glass column packed with ca. 20 ml of the appropriately silvered resin. A Milton Roy "mini-pump" was used to meter methanol to the column and the effluent was monitored by a Waters Associates Differential Refractometer (Model R403). Fractions were analyzed with an F & M 810 gas chromatograph equipped with a flame ionization detector. A 12-ft 1/4-in. stainless steel column containing 20% OV 275 on Chromosorb WAW, 100/120 was used. The XN1010 resin was ground in a Model 900 Burr Mill set at an opening of 0.06 in. (Labconco Corp., Kansas City, MO).

Preparation of Partially Silvered Resin

The ground resin was soaked in methanol (CH_3OH) for 1 hr (Caution: Heat is generated and the resin swells ca. 30%). The resin was suspended in CH_3OH 5 more times, with the solvent being rapidly decanted each time to remove the super-fines. The CH_3OH-treated resin was then washed with water (ca. 10% swelling by vol) and the addition/decantation process again repeated 5 times. The resin was allowed to settle for 15 min after each wash and was then stored overnight under the final water wash.

USA standard sieves (8 in.; Newark Wire Cloth Co., Newark, NJ) were used to separate the resin particles into various mesh sizes. About 300-ml portions of the resin were placed on the appropriate sieve and flushed with a vigorous stream of distilled water. A plastic bucket was used to collect the resin particles that passed through the sieve. After allowing the resin in the bucket to settle for 3 min, the water and super-fine resin particles (>400 mesh) were discarded. The resin remaining in the bucket was transferred to a finer sieve and the process was repeated. By this procedure, a range of mesh sizes (on 40, 40/80, 80/100, 100/120, 120/140 and 140/400) were collected. Only 40/80 and 100/120 mesh sizes were used in our study. The volume of the wet resin was measured with a graduated cylinder.

Resin containing different amounts of silver was prepared as follows: (a) for resin saturated with silver ions, a 0.2 M solution of silver nitrate ($AgNO_3$) in water was passed through a column containing the resin until the eluent was no longer acidic. The $AgNO_3$ solution and the resin were allowed to equilibrate for 1 hr, and the excess $AgNO_3$ was removed by washing with distilled water.

Gradually increasing amounts of CH_3OH in water (i.e., 25, 50, 75 and 100%) were then passed through the column. Resins prepared in this manner were assumed to be saturated (100%) with silver ions. (b) Resins not fully saturated with silver ions were prepared by a batch process. About 25 ml of the resin (in water) was transferred with 200 ml of water to a 3-necked, 500-ml round-bottomed flask equipped with an additional funnel and mechanical stirrer. The calculated amount of $AgNO_3$ (based on meq sulfonic acid/g resin) in 200 ml of water was added by drops over a 20-min period. The slurry was stirred for 1 hr and the resin was separated by vacuum filtration. After being washed with water, the resin was transferred directly into CH_3OH.

The incorporation of silver ion is described by the equation:

$$R\text{-}SO_3H + AgNO_3 \rightleftarrows R\text{-}SO_3Ag + HNO_3, \quad \text{where } R = \text{resin} \qquad [1]$$

The amount of silver incorporated on the resin could thus be determined by titration of the HNO_3 produced according to the following procedure. The aqueous eluent or filtrate was collected and diluted to a constant volume in a volumetric flask and a 50-ml portion was removed. Saturated sodium chloride was added to precipitate the excess silver ion (let stand for 15 min), and the mixture was titrated with 0.1 M sodium hydroxide in 90% CH_3OH to a phenolphthalein endpoint.

The HNO_3-containing eluents from the preparation of silver-saturated columns were titrated to determine the cation exchange capacity in meq/vol resin. The capacity of the resin in meq/ml varied little with mesh size (i.e., 40/80 at 3.6 meq/g vs 100/120 at 3.7 meq/g). The volume of the resin was measured after storing the resin in a graduated cylinder overnight. The resin was then removed by vacuum filtration, dried overnight in a vacuum oven (110 C/70 mm Hg) and weighed to determine the density in g/ml.

RESULTS

A series of partially silvered resins (54, 61, 72 and 91%) was prepared using procedure b. The theoretical percentage of silvered vs actual percentage silvered is plotted in Figure 1. From these data, the maximal percentage of silver ion that can be incorporated by a single $AgNO_3$ treatment is ca. 85%.

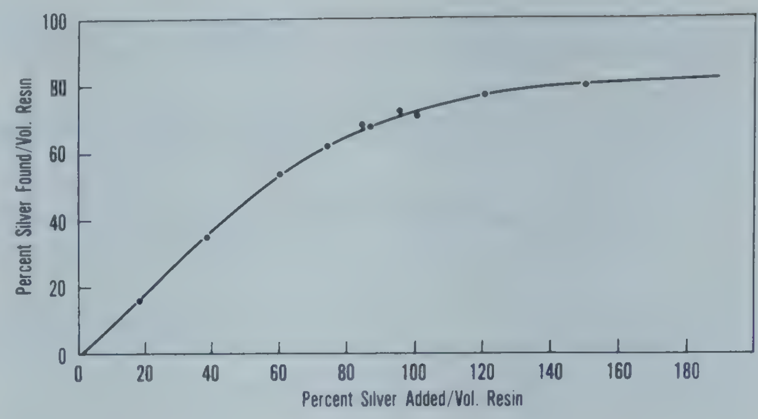

FIG. 1. Comparison of the theoretical amount of silver added by procedure b to the actual amount of silver retained on the resin (determined by titration of eluent).

Two successive batch treatments were employed when more than 75% silvering of the resin was desired. When the theoretical amount of $AgNO_3$ (equivalent to the number of meq sulfonic acid on the unsilvered resin) was used in both treatments, the same percentage of silver ion was incorporated each time. If $AgNO_3$ equivalent to the number of meq of resin unsilvered after the first treatment was used for the second silvering, the percentage of silver incorporated on the second batch was less than on the first batch. For example, to prepare a 91% silvered sample of resin, the resin was treated twice, each treatment using 100% of the theoretical quantity of silver ions (see run no. 8, Table I). Between treatments, the resin was vacuum-filtered and washed with water until neutral.

A single-treatment process was used to prepare a series of PARC columns containing 54-72% silver. A 100-mg sample of methyl linoleate was eluted with CH_3OH from each column to determine the effect of the percentage silver on peak shape and elution volume (Fig. 2). A preparative size (4 × 53 cm, 700 ml resin, 40/80 mesh) 91% PARC column was also prepared by treating the resin twice with $AgNO_3$. This column was used to separate 15- to 20-g samples of methyl 12,15-octadecadienoate-9,10-d_2 geometrical isomers as shown in Figures 3 and 4.

Table I summarizes the preparation of several partially silvered resins. Preparations 1 and 2 were saturation tests to determine the number of meq of sulfonic acid/g of resin. This number (3.6 or 3.7) was higher than the cation ex-

TABLE I

Summary of Silver.Resin Preparations

Prep. no.[a]	Resin[b] volume (ml)	Resin size (mesh)	Total volume (ml)[c]	$AgNO_3$ soln (N)	Percent silver (theor.)	Percent silver[d]	meq $AgNO_3$/g resin
1	25.1	100/120	Saturation	0.2	–	100[e]	3.7
2	28.5	40/ 80	Saturation	0.2	–	100[e]	3.6
3	27.8	40/ 80	400	0.55	60	54	1.9
4	44.6	100/120	400	0.133	73	61	2.3
5	21.3	100/120	400	0.063	99	72	2.7
6	10.0	40/ 80	30	0.436	100	71	2.6
7	9.95	40/ 80	20	0.633	100	71	2.6
8	820	40/ 80	1640	0.645	100	70[f]	2.5
	820	40/ 80	1640	0.645	100	91[f]	3.3

[a]Preparations 1 and 2 were made by method a; preparations 3-7 were 1-batch silverings; preparation 8 was a 2-batch silvering.

[b]Can be converted to g at 3.02 ml/g (40/80 mesh) and 2.83 ml/g (100/120 mesh).

[c]Milliliters of water after addition of $AgNO_3$ solution.

[d]Determined by titration.

[e]Assuming 100% silver ion incorporation.

[f]Total percentage of silver on resin after first silvering.

[g]Total percentage of silver on resin after second silvering.

change capacity of 3.1 given in the preliminary data sheet for Amberlyst XN1010 resin. Comparison of preparations 5, 6 and 7 indicates that changing the normality of the $AgNO_3$ solution in the range of 0.05-0.60 has little effect on the final percentage of silver on the resin.

DISCUSSION

Development of PARC greatly extends the application of silver resin chromatography. By varying the percentage silver on the resin, problems resulting from sample tailing and large solvent elution volumes can be minimized while an acceptable degree of separation is maintained. This concept was illustrated in the separation of the various geometrical isomers of methyl 12,15-octadecadienoate. A 91% PARC column gave almost baseline separation of these isomers. The peak shape and elution volume for the 12*c*,-15*c*-18:2 isomer is greatly improved over that previously reported for the 9*c*,12*c*-18:2 isomer.

The 91% PARC columns have been reused many times without repacking or resilvering with no deterioration in their separation capabilities. The only precautions are that the columns cannot be allowed to run dry and that large amounts of impurities cannot be present in the samples which will react with the silver ions. Compounds containing halides, e.g., can cause problems. As previously noted, a terminal olefin such as 1-hexene can be added to the methanol in order to displace highly unsaturated compounds such as polymers from the column (6). This procedure effectively cleans the column of strongly retained compounds and can be used to clean the column in order to prevent cross contamination from previous samples.

Advantages of sieving the resin wet rather than dry are that less time is required and problems with dust are eliminated. When dry sieving was used, the resin had to be sieved again after the addition of CH_3OH and H_2O because of expansion and fracturing of the resin particles and the consequent production of fines. Fines in the final mesh cut were removed to obtain the maximal flow rate through the column. A column packed with 40/80 or 80/100 mesh resin produced high flow rates at low pressure, good column capacities and adequate separation capabilities for preparative work (Figs. 3 and 4).

Use of wet resins to prepare PARC columns makes the silvering procedure somewhat empirical. The resin was kept overnight in a graduated cylinder before the volume was measured and the weight of resin/ml resin was calculated. Longer or shorter settling times would undoubtedly have changed the resin volume and affected the results. Given the errors inherent in determining the weight of the starting resin, the final percentage of silver on the resin is only relative. Despite this limitation, the method is very reproducible, and results vary by only 3-4% in the final percentage of silver on the resin.

The data in this paper explain the variations in retention of unsaturated compounds on various "saturated" silver resin columns previously prepared in our laboratory (2-8). Some of the resin columns were prepared by saturation with $AgNO_3$ (method a) and others by 3 treatments with 0.2 M $AgNO_3$ (similar to method b). Procedure b would result in silver concentrations of only 96-98% and a subsequent decrease in elution times.

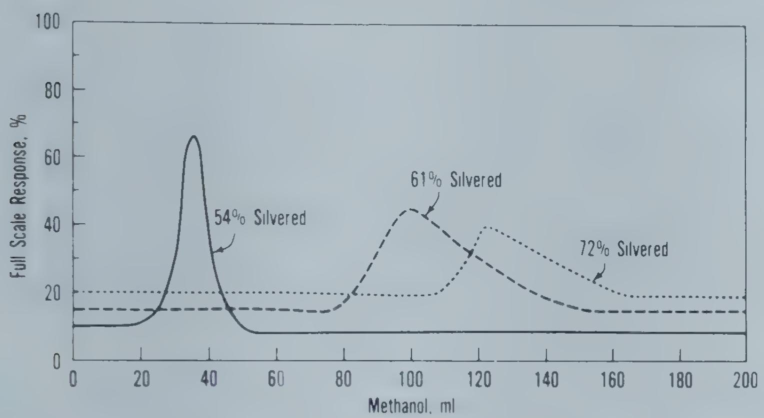

FIG. 2. Plot of the retention volume of methyl linoleate as a function of percentage silver on resin. Flow rate-0.7 ml/min CH_3OH; mesh size-40/80 or 100/120; sample size-100 mg; glass column-0.6 × 85.0 cm.

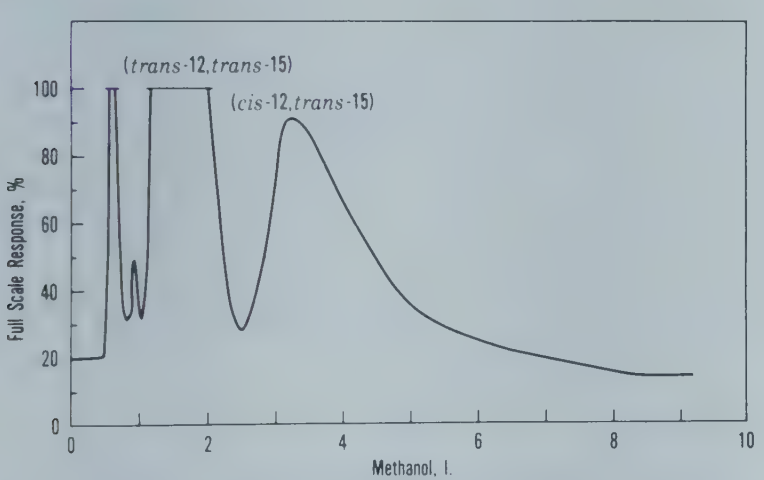

FIG. 3. Separation of 66% *trans*-12,*trans*-15- and 34% *cis*-12,*trans*-15-methyl octadecadienoate. 91%, 40/80 mesh PARC column; flow rate-10 ml/min CH_3OH; sample size-20 g.

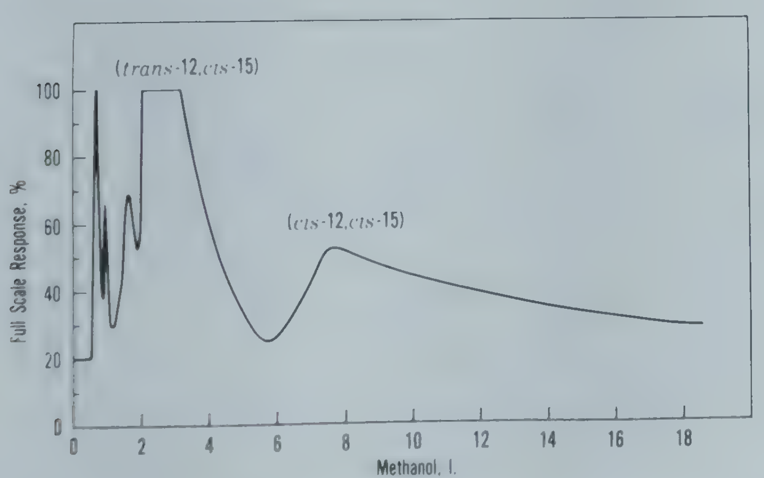

FIG. 4. Separation of 61% *trans*-12,*cis*-15- and 31% *cis*-12,*cis*-15-methyl octadecadienoate. 91%, 40/80 mesh PARC column; flow rate-10 ml/min CH_3OH; sample size-20 g.

ACKNOWLEDGMENTS

Mike Wilhelm gave technical assistance.

REFERENCES

1. Scholfield, C.R., in "Geometrical and Positional Fatty Acid Isomers," Chapt. 2, edited by E.A. Emken and H.J. Dutton, Americal Oil Chemists' Society, Champaign, IL, 1979, pp. 17-52.
2. Emken, E.A., C.R. Scholfield and H.J. Dutton, JAOCS 41:388 (1964).
3. Scholfield, C.R. and E.A. Emken, Lipids 1:235 (1966).
4. Emken, E.A., C.R. Scholfield, V.L. Davison and E.N. Frankel, JAOCS 44:373 (1967).
5. Scholfield, C.R. and T.L. Mounts, Ibid. 54:319 (1977).
6. Emken, E.A., J.C. Hartman and C.R. Turner, Ibid. 55:561 (1978).
7. Rakoff, H. and E.A. Emken, Ibid. 55:564 (1978).
8. Rohm and Haas, Preliminary Data Sheet.
9. Rakoff, H. and E.A. Emken, Lipids (in press).

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Partial Argentation Resin Chromatography (PARC): II. Separation of Saturated and Mono-, Di-, Tri- and Tetraenoic Fatty Esters

R.O. ADLOF and **E.A. EMKEN**, Northern Regional Research Center,
Agricultural Research, Science and Education Administration, USDA, Peoria, IL 61604

ABSTRACT

Partial argentation resin chromatography (PARC) was used to separate a mixture of saturated and monoenoic, dienoic, trienoic and tetraenoic fatty esters. A series of columns containing XN1010 sulfonic acid resin silvered in the range of 17-91% of theoretical (meaning 17-91% of the sulfonic acid protons were replaced by silver ions) were prepared and were used to correlate the percentage silver with the separation and the retention times of unsaturated fatty esters. *cis*-Trienoic and tetraenoic fatty esters were eluted with methanol on PARC columns containing 36 and 17% silver. Partial silvering of the resin improved peak shapes while sample elution time and elution volume were reduced. Application of PARC to the separation of mixtures of fatty acids and mixtures of triglycerides was investigated and found to be not feasible with the present system.

INTRODUCTION

Saturated silver resin chromatography has been reported to give excellent separation of saturated and monoenoic fatty esters (1-4), but the more highly unsaturated esters were found to be difficult or impossible to remove from the column by methanol (CH_3OH) elution alone (5,6). Elution of larger quantities of methyl linoleate (>20 g) from a fully saturated silver resin column required huge volumes of methanol and times approaching 1 week. A displacing agent such as 1-hexene could be used elute polyunsaturated esters, but no separation of components could then be achieved (6).

The application of partial argentation resin chromatography (PARC) has been reported for the separation of methyl 12,15-octadecadienoate isomers (7). This paper describes the extension of the PARC concept to the separation of saturated and *cis*-mono-, di-, tri- and tetraenoic fatty esters.

EXPERIMENTAL PROCEDURES

Materials

XN1010 sulfonic acid resin, 16/50 mesh, was from Rohm and Haas. The standard mixtures used were: Dienoic ester (20% saturates, 20% oleate and 60% linoleate); tri- and tetraenoic esters (5% saturates, 10% oleate, 20% linoleate, 20% linolenate and 44% arachidonate); and safflower oil methyl esters (11% saturates, 13% oleate and 75% linoleate). Saturates contain both methyl palmitate and methyl stearate. Methyl arachidonate was obtained from Hoffmann-La Roche, Inc., Nutley, NJ.

Methods

The grinding and sieving of the resins, preparations of PARC columns, silver percentage determinations (Table I) and analyses of the various eluted fractions have been described previously (7). Thin layer chromatography (TLC) (Silica Gel 60F-254; 0.25 cm; E. Merck, Darmstadt, Germany) was used to determine the effect of silvered resin on fatty acids and triglycerides. Samples were developed using a 50:50 mixture of petroleum ether (P.E.) and diethyl ether (Et_2O) and visualized with sulfuric acid and charring. Di- and tetraenoic standard mixtures (100-mg) were separated on an 85 × 0.6 cm glass column packed with 20-ml samples of 17-54% silvered (meaning 17-54% of the resin sulfonic acid protons were replaced by silver ions) resins. Safflower oil methyl esters (∿20 g) were separated by a 4 × 53 cm glass column containing 700 ml of 40/80 mesh, 91% silvered resin.

RESULTS

Figures 1-3 illustrate the separation of 100-mg samples of fatty esters on 54, 36 and 17% PARC (silvered) columns.

TABLE I

Summary of Silver Resin Preparations

Prep. no.[a]	Resin (ml)	Resin size (mesh)	Total volume[b] (ml)	$AgNO_3$ soln. (N)	Percent silver (theor.)	Percent silver[c]	meq $AgNO_3$/g resin
1	27.8	40/ 80	400	0.55	60	54	1.9
2	26.3	80/100	400	0.040	39	36	1.3
3	23.2	80/100	400	0.016	18	17	0.6
4	36.0	16/ 50	Saturation[d]	0.16	100	100	3.3
5	820	40/ 80	1640	0.645	100	70[e]	2.5
			1640	0.645	100	91[f]	3.3

[a]Runs 1-4 are one-batch silverings; run 5 is one batch silvered twice.
[b]Milliliters of water after addition of $AgNO_3$ solution.
[c]Determined by titration.
[d]Unground resin; washed with 0.16 N $AgNO_3$ until saturated.
[e]Total percent silver on resin after first silvering.
[f]Total percent silver on resin after second silvering.

The separation of safflower oil methyl esters is illustrated in Figure 4. About 14 g of 99+% pure methyl linoleate was isolated from each 20-g sample. A 25-ml sample of unground (16/50 mesh), 100% silvered resin was also prepared to determine if the resin could be used as received. Separations were found to be poor (Fig. 5). Although saturated and monoenoic esters could be partially separated, the dienoic esters began to elute from the column as part of the monoenoic ester peak.

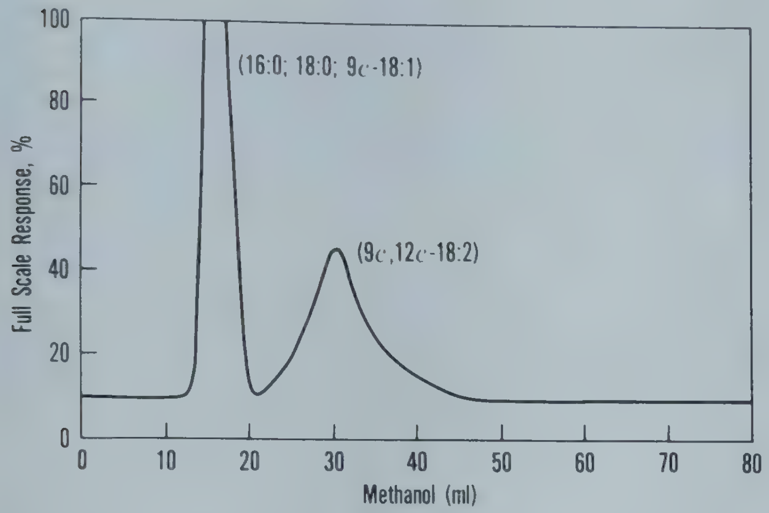

FIG. 1. Separation of the dienoic ester standard on an 85 × 0.6 cm 54% PARC column. Flow rate: 0.55 ml CH_3OH/min; 40/80 mesh; sample size: 100 mg.

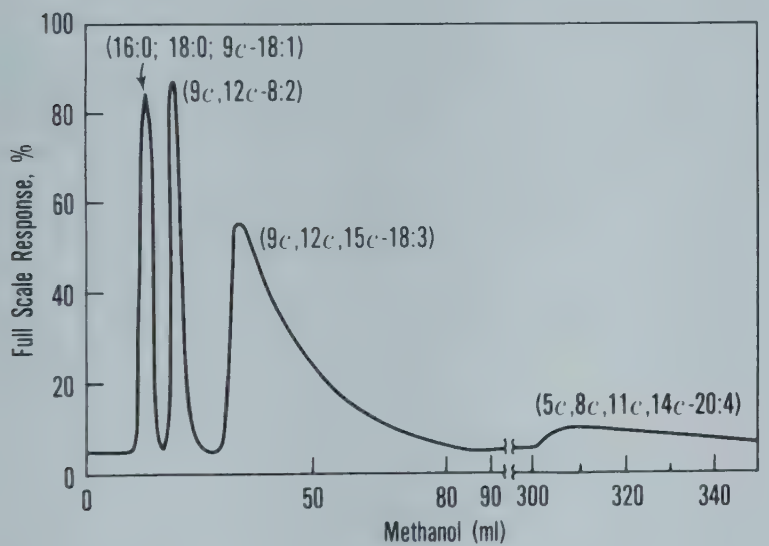

FIG. 2. Separation of the tetraenoic ester standard on an 85 × 0.6 cm 36% PARC column. Flow rate: 0.40 ml CH_3OH/min; 80/100 mesh; sample size: 100 mg.

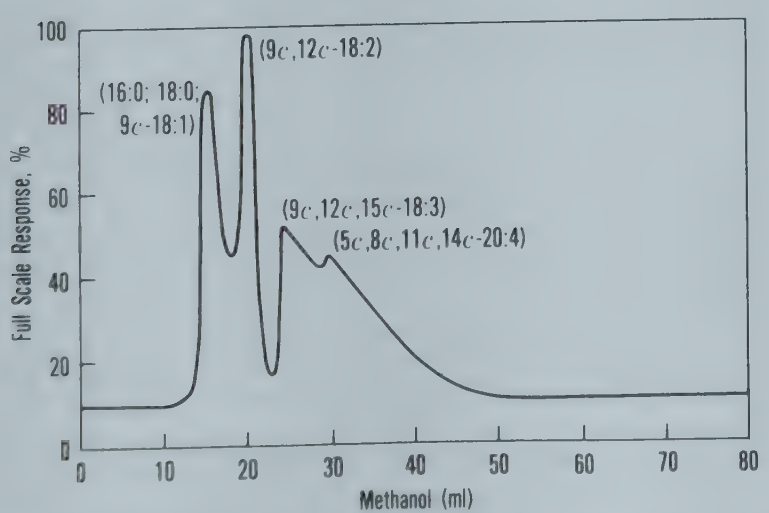

FIG. 3. Separation of tetraenoic ester standard on an 85 × 0.6 cm 17% PARC column. Flow rate: 0.19 ml CH_3OH/min; 80/100 mesh; sample size: 100 mg.

The feasibility of separating mixtures of fatty acids or triglycerides using PARC was also investigated. Small samples of oleic acid (50 mg) were placed in vials containing ca. 10 ml of methanol and either 5 ml of 100% silvered or 50% silvered resin. The vials were left at room temperature overnight and the supernatant was tested by thin layer chromatography (TLC) for the presence of methyl oleate. A significant amount of methyl oleate (20-30%) was found, thus indicating esterification had occurred. Similar tests using triolein also produced methyl oleate (<5% for 100% silvered; 15-20% for 50% silvered). Thus, both esterification and transesterification occurred, even when silver-saturated resin was used. Apparently these reactions were catalyzed by traces of free sulfonic acid groups remaining on even the 100% silvered resin.

DISCUSSION

The applications of silver resin chromatography have been expanded by the PARC concept. Polyenoic esters can now be separated by control of the percentage of silver on the resin. The effect of partial silvering is illustrated in Figures 1-3. Dienoic esters can be separated from monoenoic and saturated esters using a 54% PARC column. A 36% PARC column can be used to isolate both dienoic and trienoic esters and tetraenoic esters can be eluted from a 16% PARC column. The poor separation of trienoic and tetraenoic esters on the 16% PARC column may indicate the limit to which the percentage of silvering of the resin can be lowered and usable separation still be achieved. However, a 36% PARC column could be used to selectively separate all *cis*-dienoic, trienoic and tetraenoic fatty esters if the tetraenoic fatty esters were eluted with 1-hexene. These separations are currently impractical using a saturated silver resin column (5,6).

Since flow rate,percentage of silver and column length can be varied, the parameters used in this paper provide only general guidelines for the preparation of suitable columns. Optimization of the parameters for specific separations was not investigated. Consequently, the use of high flow rates with columns containing the highest possible percentage of silver (where the sample can still be eluted) was the best strategy for preparative separation of polyenoic fatty esters.

Elution volume for saturated fatty esters was essentially constant despite large variations in the percentage of silver. Only with the unground resin did the saturates begin to elute earlier (Fig. 5), perhaps because of channeling. Thus

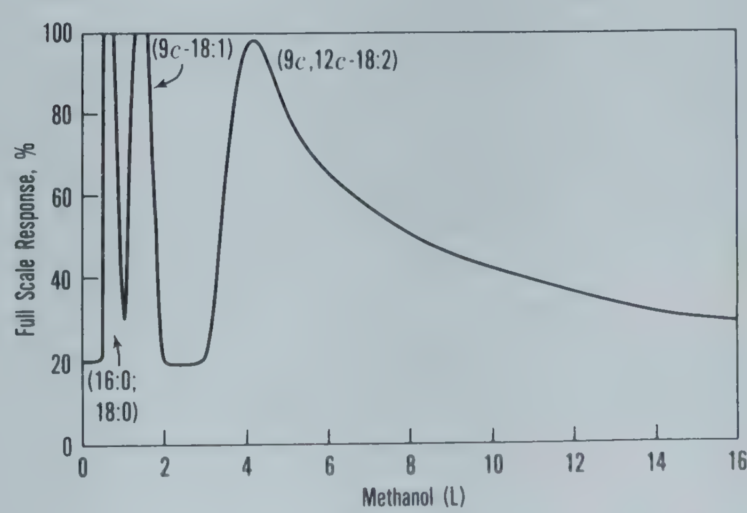

FIG. 4. Separation of safflower oil methyl esters on a 53 × 4 cm 91% PARC column. Flow rate: 10 ml CH_3OH/min for 4 liters, then 12 ml/min; 40/80 mesh; sample size: 20 g.

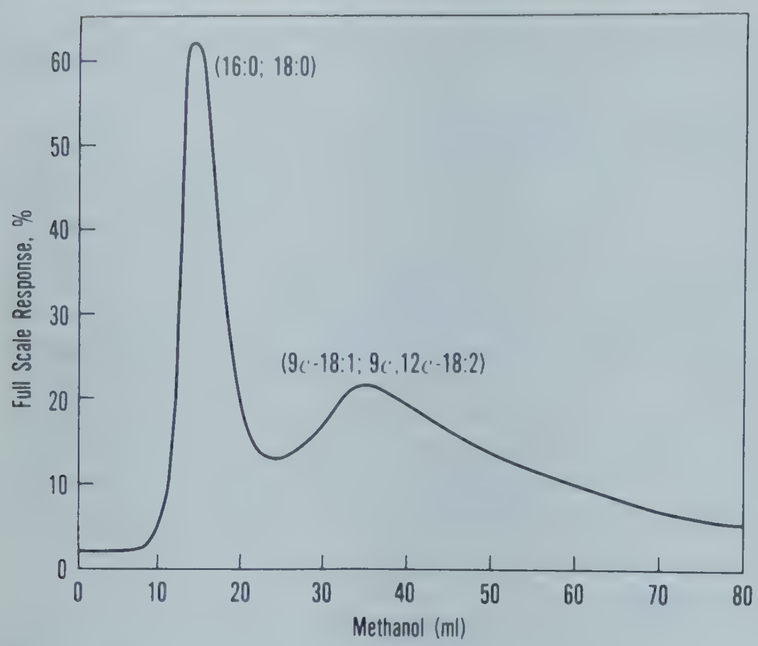

FIG. 5. Separation of the dienoic ester standard on an 85 × 0.6 cm 100% silvered column. Flow rate: 0.27 ml CH_3OH/min; 16/50 mesh; sample size: 100 mg.

grinding and sieving of the resin are necessary for good sample resolution. A column packed with 40/80 mesh resin is a good choice. With this mesh size, high flow rates (at low back pressure) and capacities can be maintained while channeling is kept to a minimum.

The advantages of PARC over other separation techniques are numerous. Unlike previously employed resins such as XN1005, the XN1010 resin is commercially available. Older XN1005 saturated silver resin columns have lasted for more than 10 years, thus demonstrating their durability. In general, the columns can be reused indefinitely. The silver incorporated onto the resin can be easily recovered if necessary by regenerating the resin with nitric acid. Polyunsaturated esters or tightly bound impurities were previously shown to be displaced using a 10-20% solution of 1-hexene in methanol (6). The capacities of the silver resin columns are high compared to silver-silica gel columns, since a 4 × 53 cm, 91% PARC column can easily separate 20-g samples of safflower oil methyl esters, and samples twice as large can be used if only the dienoic esters are to be isolated. The PARC concept allows a column to be tailored to the specific separation needed.

Although this method is not applicable to fatty acids and triglycerides, preparative separation of large quantities of saturated and *cis*-mono-, di-, tri- and tetraunsaturated fatty esters is now feasible. The PARC concept should be a useful technique for those laboratories requiring large, highly pure samples of these important compounds.

Since this work was completed and reviewed by *JAOCS*, the authors have become aware of a patent issued in February 1980 to the Procter and Gamble Company which described some of the concepts of partial argentation resin chromatography used in this paper (8).

REFERENCES

1. Emken, A.E., C.R. Scholfield and H.J. Dutton, JAOCS 41:388 (1964).
2. Scholfield, C.R. and E.A. Emken, Lipids 1:235 (1966).
3. Emken, E.A., C.R. Scholfield, V.L. Davison and E.N. Frankel, JAOCS 44:373 (1967).
4. Scholfield, C.R., and T.L. Mounts, Ibid. 54:319 (1977).
5. Wurster, C.F., Jr., J.H. Copenhaven, Jr. and P.R. Shafer, Ibid. 40:513 (1963).
6. Emken, E.A., J.C. Hartman and C.R. Turner, Ibid. 55:561 (1978).
7. Adlof, R.O., Henry Rakoff and E.A. Emken, Ibid., submitted.
8. Lubsen, T.A. and G.A. Maag, U.S. Patent 4,189,442 (1980).

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Electron Microscopy of Soybean Lipid Bodies

C.W. BAIR[1] and **H.E. SNYDER,**[2] Department of Food Technology,[3]
Iowa State University, Ames, IA 50011

ABSTRACT

Soybean lipid bodies in situ are small (0.2-0.5 μm in diameter) and have an affinity for plasmalemma, protein bodies, endoplasmic reticulum and other cell organelles but not for mitochondria or nuclei. Isolated lipid bodies contain 15% protein after extensive washing and have densities ranging from less than 1.0066 to 1.0788. We concluded that lipid bodies are surrounded by a specific delimiting membrane.

INTRODUCTION

Compared to the substantial economic importance of soybean oil, there is surprisingly little research that has been reported on the lipid-containing structures of soybeans. Bils and Howell (1) described lipid granules in electron micrographs of developing soybean cotyledons. Saio and Watanabe (2) and Wolf and Baker (3,4) also have made electron microscopic studies of soybeans and noted the lipid-containing organelles. To our knowledge, Kahn et al. (5) have published the only report on isolation and analysis of the lipid-containing organelles from soybeans.

There is considerable literature on lipid-containing organelles from other seeds and from other plant tissues (6-11), and a controversy exists about nomenclature of the lipid-containing organelles. The most common name is spherosome, but this term also has been associated with enzymatically active organelles such as glyoxysomes and lysosomes. Although the spherosome name has been used for lipid-containing organelles in soybeans (2-4), because of the controversy about spherosomes in other tissues and because of the lack of descriptive content (spherosome = round body), we prefer the term "lipid body" for the oil-rich organelles and will refer to them as lipid bodies in this paper.

MATERIALS AND METHODS

Amsoy 71 seed-grade soybeans were the experimental material for all micrographs and isolations of lipid bodies.

Sample Preparation for Transmission Electron Microscopy (TEM) and for Scanning Electron Microscopy (SEM)

For TEM, samples were cut into small pieces and fixed overnight in 3% glutaraldehyde-1.5% paraformaldehyde in 0.1 M phosphate buffer at pH 7.1 and 4C. The pieces were then rinsed for 3 20-min periods in phosphate buffer and postfixed for 2 hr in 2% osmium tetroxide (same buffer and pH) at room temperature. Fixation was followed by 3 20-min phosphate buffer rinses, dehydration through an acetone series, infiltration with propylene oxide and final infiltration and embedding in Spurr's resin (12). Sections having silver to gold interference colors were cut with glass knives using an LKB Ultratome III. Sections were picked up on copper grids and stained with methanolic uranyl acetate (13) and lead citrate (14). Samples were examined on a Hitachi HU-11C transmission electron microscope operated at an accelerating voltage of 50 kV.

For SEM the samples, after acetone dehydration, were frozen in parafilm pillows filled with absolute acetone and cryofractured in liquid nitrogen using a razor blade held in a hemostat. Fractured pieces were dropped into acetone to thaw, infiltrated with Freon TF (113) and critical-point dried using liquid carbon dioxide. Samples were mounted on brass discs with silver conductive paint, lightly coated with carbon and ca. 10 nm of gold in a Varian VE-30 vacuum evaporator. Samples were examined on a JEOL JSM-35 scanning electron microscope operated at 15 kV with a beam current of 80 μA.

Isolated lipid bodies were embedded in 2% agar and small cubes (ca. 1 mm^3) were taken through the fixation, dehydration and embedding procedures already outlined for TEM and SEM.

Isolation of Lipid Bodies

The methods of Jacks et al. (11), Kahn et al. (5) and Yatsu and Jacks (15) were used to separate a floating "fat pad" from the other soy constituents. After preliminary experiments, the Yatsu and Jacks method (15) was chosen for all subsequent isolations.

The floating layers were dialyzed for 2 days against distilled water at 4 C to remove salt or sugar before analysis. Samples for analysis were freeze-dried after dialysis.

Protein Analysis

The AOAC (16) micro-Kjeldahl procedure was used for digestion except that 0.2 g cupric selenite served as catalyst in place of mercuric oxide. A Lab Con Co micro-distillation unit was used to recover ammonia, and total nitrogen was multiplied by 6.25 to convert to protein.

Lipid Analysis

Crude lipid was extracted with hexane in a Goldfisch apparatus by using method (30-20 of AACC (17). For both protein and lipid analyses, duplicate samples were run and the results reported are the averages.

Density Gradient Centrifugation

The isolated lipid bodies were analyzed by both continuous and discontinuous sucrose density gradients. The lipid body isolate was suspended in distilled water and carefully layered on top of the gradients. The discontinuous gradient consisted of zones of 0.05 M, 0.1 M, 0.2 M, 0.4 M and 0.6 M sucrose. The continuous gradient ranged from 0.1 M to 0.6 M sucrose. The centrifugations were done at 4 C for 7 hr at 100,000 × g in a Beckman model L3-50 ultracentrifuge with a SW 41 rotor.

Tryptic Hydrolysis

Agar-embedded lipid bodies were placed in vials containing 5 ml of a trypsin solution (5 mg of trypsin in 0.001 N HCl) and incubated at 37 C for 12 hr.

Acid Phosphatase

Localization of acid phosphatase activity in isolated lipid bodies was done using a modification of the Barka and Anderson procedure (18). Aldehyde-fixed tissues were

[1] Frito-Lay Div., PepsiCo Inc., 900 North Loop 12, Irving, TX 75061.

[2] Department of Horticultural Food Science, Univ. of Arkansas, Fayetteville, AR 72701.

[3] Journal Paper No. J-9742 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Project No. 2159.

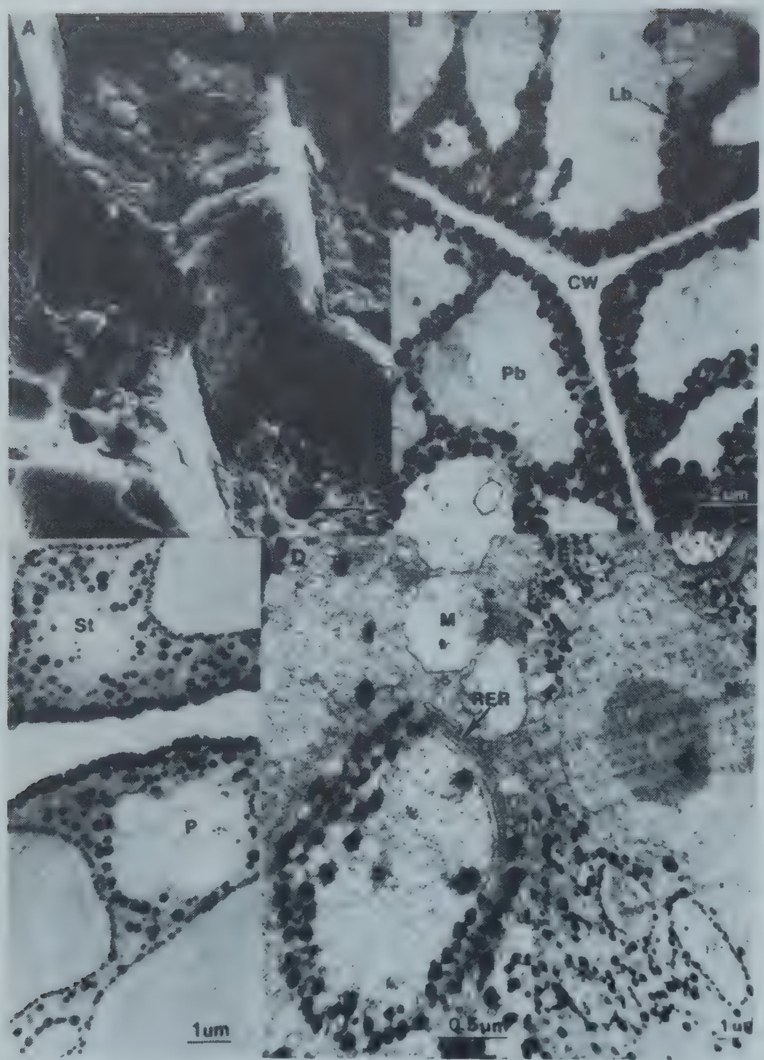

FIG. 1. Electron micrographs of lipid bodies in situ. A: SEM micrograph of freeze-fractured soybean cotyledon; B: TEM micrograph of soybean cotyledon showing lipid bodies densely packed; C: TEM micrograph showing lipid bodies concentrated at protein body and cell membranes but not at amyloplast membrane; D: TEM micrograph of soybean hypocotyl showing lipid bodies concentrated at rough endoplasmic reticulum but not at mitochondrial membrane; E: TEM micrograph of soybean hypocotyl showing lipid bodies concentrated around unidentified organelles but not around the nucleus. Labels are: Pb = protein body, Lb = lipid body, CW = cell wall, St = starch granule, P = amyloplast, M = mitochondrion, RER = rough endoplasmic reticulum and N = nucleus.

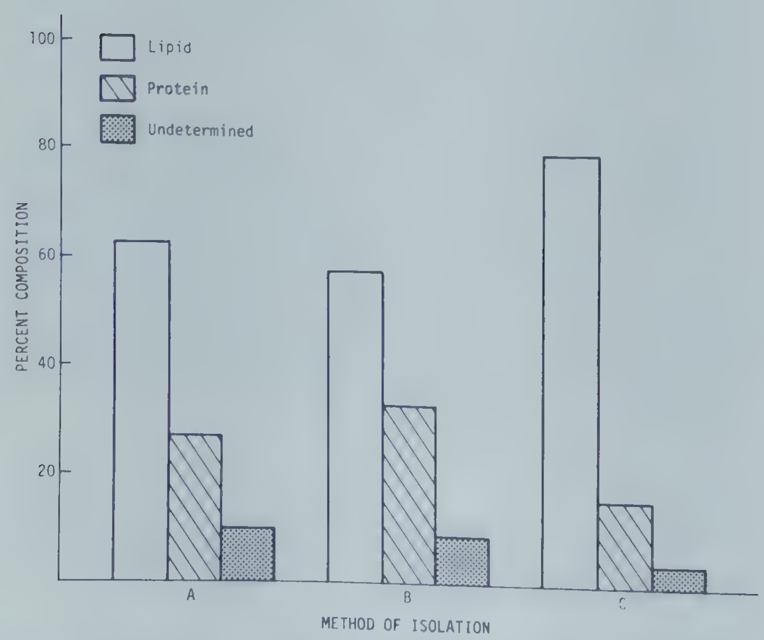

FIG. 2. Effect of the isolation method on the composition of the isolated lipid bodies. A is the Jacks et al. procedure (11), B is the Kahn et al. procedure (5) and C is the Yatsu and Jacks procedure (15).

placed in 5 to 10 ml of the reaction mixture (previously adjusted to pH 5.5) and incubated at 37 C for 30, 60, 90 and 120 min. These controls were used: (a) tissues incubated in the reaction mixture with no substrate (sodium *beta*-glycerol phosphate), (b) tissues incubated in complete reaction mixture plus 0.01 M sodium fluoride, and (c) tissues boiled for 15 min in water before incubating in the reaction mixture.

RESULTS AND DISCUSSION

Figure 1 shows lipid bodies in situ. When viewed by SEM (Fig. 1A), the lipid bodies were enmeshed in what has been referred to as a cytoplasmic network (4) presumably composed of protein. The spaces between protein bodies in cotyledon cells were filled with the lipid body network.

When viewed by TEM (Fig. 1B), lipid bodies also filled the spaces between protein bodies in some cotyledon cells and were discrete particles, ca. 0.2-0.5 μm in diameter, with no cytoplasmic network. In other cotyledon cells, lipid bodies were less concentrated (Fig. 1C) and definite patterns of concentration were discerned. Lipid bodies showed an affinity for protein bodies and for the cell membrane (cell wall) but not for amyloplasts or mitochondria. Figures 1D and 1E are from soybean hypocotyl and show further distinctions in affinities of lipid bodies for other cell organelles. In Figure 1D, lipid bodies are clustered about rough endoplasmic reticulum, and in Figure 1E, the lipid bodies show an affinity for unidentified organelles (possibly glyoxysomes) but no affinity for the nucleus.

Webster and Leopold (19) noted that lipid bodies from soybeans had affinity for protein bodies and for cell membrane (plasmalemma), and Jelsema et al. (10) observed that lipid bodies from wheat had an affinity for aleurone grains. The lack of affinity between lipid bodies and amyloplasts, mitochondria or nuclei suggested that lipid bodies adhered to those cell organelles having a single-unit membrane but did not adhere to organelles with a double-unit membrane.

Yatsu and Jacks (15) showed that the membrane surrounding peanut lipid bodies was a half-unit membrane and, when isolated, could recombine to form a unit membrane. Our observations show some differences between unit membranes on the basis of affinity for lipid bodies.

To further our understanding of the strucure and composition of soybean lipid bodies, we isolated them from mature cotyledons. Three isolation procedures were used (5,11,15), and the compositions of the fat pads isolated by these procedures are shown in Figure 2. We found more protein (32% vs 10%) and less lipid (58% vs 89%) than did Kahn et al. (5) who made the only other analysis of soybean lipid bodies of which we are aware. A possible explanation for the differences in analyses is that they isolated lipid bodies in 0.5 M sucrose and corrected dry weights for estimated sucrose content. Their lipid extraction was done with ethanol/ether/chloroform (2:2:1), which would have extracted sucrose and may have caused an overestimation of lipid, because they took the weight of the solvent-free extract to be lipid.

Jacks et al. (11) found 98.1% lipid in their isolated lipid body fraction from peanuts. The average diameter of peanut lipid bodies is greater than 1 μm. Since lipid bodies from peanuts are larger than those from soybeans, the larger size may have contributed to a cleaner centrifugal separation and a higher lipid content. Using the Jacks et al. procedure (11) on soybeans, we found 63% lipid and 27% protein.

The increased protein in our preparations may be a direct consequence of the small size of the lipid bodies and the corresponding increase in surface area. For example,

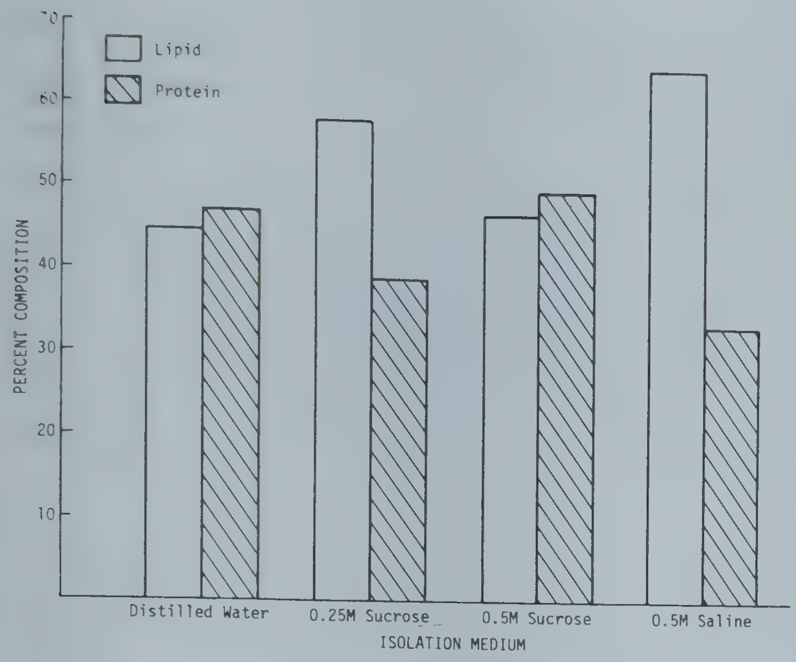

FIG. 3. Effect of the isolation medium on the composition of the isolated lipid bodies. The Jacks et al. method (11) was used for all isolations.

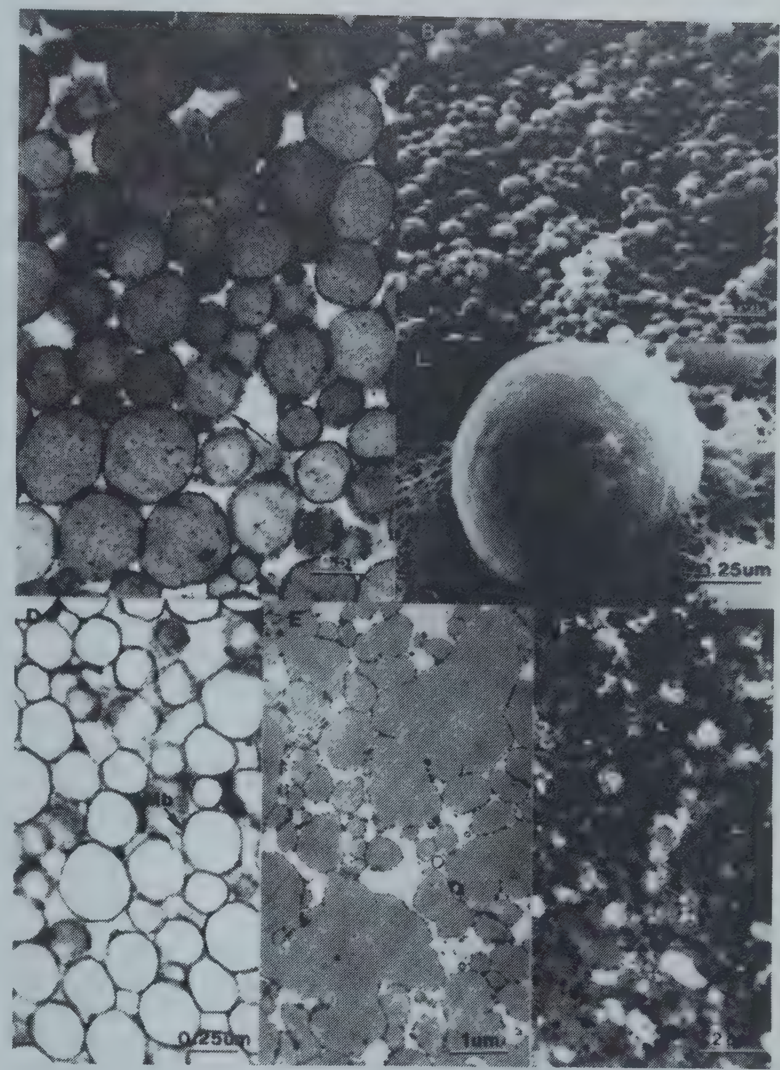

FIG. 4. Electron micrographs of isolated lipid bodies. A: TEM micrograph of isolated lipid bodies; B: SEM micrograph of isolated lipid bodies showing little cytoplasmic network; C: SEM micrograph of isolated lipid body at high magnification; D: TEM micrograph of isolated lipid bodies after extraction with hexane; E: TEM micrograph of isolated lipid bodies after treatment with trypsin; F: TEM micrograph of centrifugal pellet obtained during isolation of lipid bodies. Label: Mb = membrane.

lipid bodies with a diameter of 0.2 μm compared to a diameter of 2 μm would have a 10-fold increase in surface area and presumably a 10-fold increase in protein content/unit vol of lipid.

To investigate the influence of the isolation medium on the composition of lipid bodies, we used water, 0.25 M sucrose, 0.5 M sucrose and 0.5 M saline to isolate lipid bodies, but all isolations were done by the Jacks et al. (1) procedure. The results in Figure 3 show that the most lipid and the least protein were obtained with 0.5 M saline. Because 0.5 M saline was the isolation medium used by Yatsu and Jacks (15), we did the remaining isolations of lipid bodies using their procedure.

Our analyses showed that lipid bodies contained considerable protein, yet TEM micrographs showed a clean-looking preparation (Fig. 4A) very similar to those for peanut lipid bodies (11,15). The protein must adhere tightly to the lipid bodies because it survives extensive (9X) washing.

The amount of floating fat pad recovered was dependent on the ratio of soybeans to isolating medium. With a ratio (by weight) of 1:3 or 1:4, an appreciable fat pad was separated, but if the ratio was 1:10, no floating fat pad was evident. One possible reason for the difference was that at soybean-to-isolating-medium ratios of 1:3 or 1:4, the protein bodies might remain intact, and less protein would be available to interact with lipid bodies. At a ratio of 1:10, protein bodies disintegrate and the soluble protein might adhere to lipid bodies, thereby increasing their density and decreasing the floating fat pad.

We observed by SEM that the cytoplasmic network evident in Figure 1A is greatly decreased in lipid body isolates (Fig. 4B), but some adhering material is visible at higher magnification (Fig. 4C).

When isolated lipid bodies were extracted with hexane (Fig. 4D), we could observe membranes that were about half the thickness of a unit membrane. This observation is the same as that made by Yatsu and Jacks (15) for isolated peanut lipid bodies.

To learn more about the distribution of densities in lipid body isolates, we centrifuged them in continuous and discontinuous sucrose density gradients. In continuous gradients, an even distribution was found. In discontinuous gradients, lipid bodies were found floating at all boundaries. Thus, the isolated lipid bodies have a wide range (<1.0066-1.0788) of evenly distributed densities.

Even when a floating fat pad was evident, we found considerable lipid in the supernatant fluid and in the centrifugal pellet during isolation in 0.5 M NaCl (D_{20}^{20} = 1.0205). The lipid bodies present in the centrifugal pellet (Fig. 4F) were considerably smaller than normal (most were 0.1 μm or less in diameter). Smaller lipid bodies, if coated with protein, would be of higher density than larger lipid bodies and might be expected to sediment in a centrifugal field.

Evidence for a protein component essential to the integrity of the lipid body membrane came from experiments on tryptic hydrolysis of lipid bodies. Figure 4E shows the results of subjecting lipid bodies to tryptic hydrolysis. Some breakdown of membranes and coalescence of lipid bodies were evident. Still the membranes did not break down entirely to give spherical lipid droplets. We interpret Figure 4E to be further evidence for the existence of membranes surrounding lipid bodies and to be evidence that those membranes contain protein as an essential part of their normal structure—not randomly adhering protein.

Analyses for acid phosphatase showed no activity in any of the lipid body preparations, but positive evidence of activity (deposition of lead salts) was found in soybean protein bodies.

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REFERENCES

1. Bils, R.F., and R.W. Howell, Crop Sci. 3:304 (1963).
2. Saio, K., and R. Watanabe, Agric. Biol. Chem. 30:1133 (1966).
3. Wolf, W.J., and F.L. Baker, Cereal Sci. Today 17:123 (1972).
4. Wolf, W.J., and F.L. Baker, Cereal Chem. 52:387 (1975).
5. Kahn, V., R.W. Howell and J.B. Hanson, Plant Physiol. 35:854 (1960).
6. Sorokin, H.P., and S. Sorokin, Protoplasma 62:216 (1966).
7. Yatsu, L.Y., T.J. Jacks and R.P. Hensarling, Plant Physiol. 48:675 (1971).
8. Martile, P., and J. Spichiger, Z. Pflanzen Physiol. 58:277 (1968).
9. Frey-Wyssling, A., E. Grieshaber and K. Mühlethaler, J. Ultrastruct. Res. 8:506 (1963).
10. Jelsema, C.L., D.J. Morre, M. Ruddat and C. Turner, Bot. Gaz. 138:138 (1977).
11. Jacks, T.J., L.Y. Yatsu and A.M. Altschul, Plant Physiol. 42:585 (1967).
12. Spurr, A.R., J. Ultrastruct. Res. 26:31 (1969).
13. Stempack, J.G., and R.J. Ward, J. Cell Biol. 22:697 (1964).
14. Reynolds, E.S., Ibid. 17:208 (1963).
15. Yatsu, L.Y., and R.J. Jacks, Plant Physiol. 49:937 (1972).
16. Official Methods of Analysis, 11th Edition, Association of Official Analytical Chemists, Washington, DC.
17. AACC Approved Methods, 7th Edition, American Association of Cereal Chemists, St. Paul, MN.
18. Barka, T., and P.J. Anderson, J. Histochem. Cytochem. 10:741 (1962).
19. Webster, B.D. and A.C. Leopold, Am. J. Bot. 64:1286 (1977).

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Triglyceride Analysis with Glass Capillary Gas Chromatography

K. GROB, Jr., H.P. NEUKOM and **R. BATTAGLIA,**
Kantonales Laboratorium, PO Box, 8030 Zürich, Switzerland

ABSTRACT

The analyses of triglycerides on capillary columns is reported. Applications in which this technique can be used include: rapid identification of fats and oils, measurement of butter fat or coconut oil content in margarine or chocolate, monitoring of processes such as fractionation, transesterification or heat treatment. Although separation of all isomers within a group of triglycerides with identical carbon numbers is not usually feasible by gas chromatography (GC) alone, the resolution obtained with capillary columns gives more information than that obtained with packed column GC. The conditions used in this work are described. Persilylated columns coated with nonpolar gum phases could be used for ca. 1 yr with hundreds of injections.

INTRODUCTION

Fats and oils are largely characterized by highly developed procedures to analyze the fatty acid methyl esters (FAME), (1). These relatively volatile compounds are amenable to gas chromatography (GC) analyses and give good fingerprint chromatograms for different fats and oils. Simultaneously, this GC method has been widely used to determine the *trans* isomers of 18:1 fatty acids produced by hydrogenation or prolonged heat-treatment of fats or oils, despite the fact that the *cis* and *trans* isomers cannot be satisfactorily separated even with capillary columns (2). HPLC on $AgNO_3$-coated silica gel gives better results for these separations (3).

The analysis of triglycerides complements the chromatography of FAME if it is used for identification purposes or to measure butterfat or coconut oil content. The method is extremely simple, since sample preparation consists of merely diluting the fat with a solvent.

The technical aspect of the GC analysis requires some attention. As soon as a suitable column and an appropriate injection technique is used, the method becomes routine, even though molecular weights of some triglycerides exceed 1000 daltons.

Triglyceride analysis on packed columns has been done for nearly 20 years (4,5) and is a rapid, routine method (6). On nonpolar stationary phases, it is possible to separate the triglycerides according to their total carbon number (not including the odd-numbered ones). An attempt has been made to separate triglycerides based on their degree of unsaturation (7). However, the Silar 10C-phase used for this purpose has to be operated at its temperature limit.

The use of capillaries provides the well known advantages of increased resolution and relatively short analysis time. Little has been published on this technique: Schomburg et al. (8) shows some chromatograms; Schulte reports some applications (9); and a recent paper (10) shows a number of capillary GC runs on very short columns and gives information on the column and chromatographic conditions in detail. Although separations on 4-6-m capillaries are much better than on packed columns, we believe that considerably more information could be obtained using 15-20-m columns.

Apolar stationary phases such as OV 1 and 101, SE 30 and 52 separate the triglyceride peaks according to the total number of carbon atoms. The composition of these groups is complex; (a) there are many isomers of the same molecular weight with different combinations of various fatty acids, e.g., 12-12-12, predominate in coconut oil (11), which is well separated from 4-14-18, predominate in butter fat (12) (Fig. 1); (b) triglycerides composed of the same fatty acids may exist as stereoisomers, differing in the position of the fatty acids on the glyceride moiety; (c) a considerable proportion of the fatty acids is unsaturated, giving a large number of possible isomers differing in the number of double bonds as well as their distribution in the triglyceride molecule.

With the separation technique used presently, it is impossible to separate more than a small number of these isomeric triglycerides within one run. Nevertheless our experiments have shown that characteristic peak patterns are obtained for most fats.

At this point, it might be asked why we prefer nonpolar phases over polar ones. Nonpolar phases have good selectivity for structural and stereoisomers as they are characteristic in butter fat e.g., But the separation of unsaturated species is less satisfactory: resolution may be quite high, but peaks of different identity are mixed, not grouped according to the number of double bonds as on polar phases. There is little chance to develop simple rules to interpret or predict the appearance of unsaturated trigly-

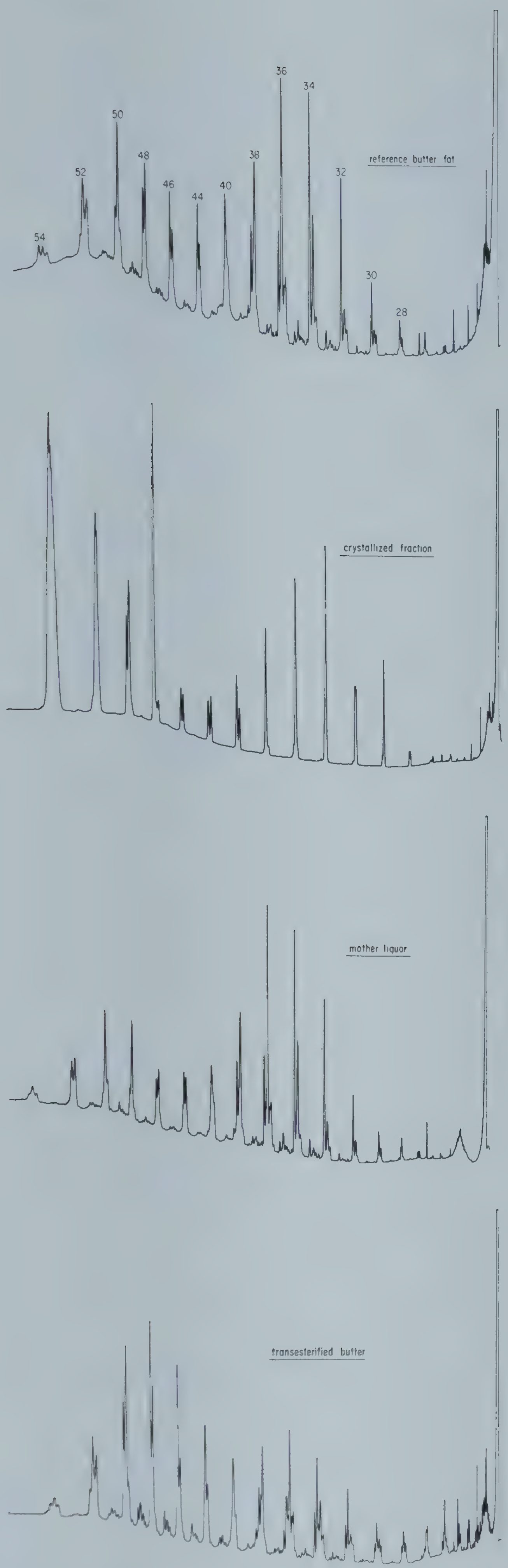

FIG. 1. Chromatograms of untreated butter, a crystallized fraction, the mother liquor of the same fractionation and the transesterified fat. Note the strong changes within each group of triglycerides whereas the molecular weight distribution did not change accordingly. Column: 14 m/0, 3 mm/0.08 μm OV-1, 0.8 at H_2 as carrier gas, temperature program 4 C/min (240-340 C). Cold on-column injection at 60 C (0.5 μl, hexane as solvent).

cerides in a chromatogram. Although differences among various plant oils and fats can be observed, they are difficult to characterize. The answer to the given question is pragmatic: we have not been able to produce polar columns with comparable lifetimes, similar efficiency and the same inertness as nonpolar columns.

PROCEDURE

Equipment: gas chromatograph: Model 4160, (Carlo Erba, Strumentazione, Italy) equipped with FID and 2 injectors, a vaporizing split/splitless and a cold on-column injector.

The fat was diluted 1:1000-1:10,000 in hexane. For butter fat or margarine it was not necessary to dry this solution even when it turned turbid from humidity. This sample (0.5-1 μl) was introduced by rapid movement of the plunger directly into the oven-thermostated column using the cold on-column injection technique. During the sampling the column was kept at 50-60 C to control the evaporation speed of the solvent. After 20-30 sec (after which most of the solvent is evaporated) the column was rapidly heated to a temperature between 240 and 300 C, immediately starting a temperature program of ca. 4 C/min to 340-370 C (temperatures depending on the kind of fat and the film thickness of the stationary phase).

Capillary columns (15 m x 0.30 mm) were used with persilanized support, coated with a film of 0.12 μm of OV 1. The inlet pressure of the carrier gas (hydrogen) was 0.7 atm. The FID fuel gases had to be adjusted to produce a minimum of sensitivity drift during the temperature program (13), i.e., 40 ml/min of hydrogen and 500 ml/min air for the instrument used.

RESULTS AND DISCUSSION

Application in Food Analysis

Measurement of butter fat and coconut oil content. According to the literature, the quantitative composition of some special fat mixtures, such as peanut/coconut oil have been determined on the basis of triglycerides (14). However, the method still is not used for several applications where it might be very useful: butter fat content in margarines or chocolate products have usually been determined using methylbutyrate as a marker; methyllaurate was used for coconut oil and palm kernel fat. The butyric acid content in triglycerides of milk of different origin and different seasons varies considerably, which requires the use of large tolerance limits. Our faster and more precise method quantitates typical triglyceride peaks: the fat is dissolved in hexane; a known amount of an internal standard, e.g., the alkane C_{40} (eluting between the triglyceride groups 34 and 36), is added (usually 3%/100% butter); and quantitation is carried out on the basis of peak heights calibrated for a number of peaks of pure butter fat. Within certain limits butter fat and coconut oil can be quantitated even when they are mixed. Figure 2 shows that a minimum of 10% coconut oil in butter or 20% butter in coconut oil is required for a reasonable determination. If they occur separately, much smaller fractions of the content can be quantitated easily.

Fractionated fats. Fractionation of a fat can be more sensitively monitored on the triglyceride basis than by the classical analysis of FAME, since it is a direct analysis of the species affected by such processes (e.g., in a crystallized fraction, where tripalmitin is strongly enriched). The FAME analysis of the methylpalmitate reflects this incorrectly, because other triglycerides, also containing palmitic acid, are not enriched at the same time. Figure 3 gives an example of a fractionated margarine; Figure 1 shows

a crystallized butter, which is used in the baking industry. In both cases, the distribution of the molecular weights of the triglycerides (as observed by packed column GC) is changed. For the butter fat an increase in the proportion of larger triglycerides is observed for the crystallized fraction. However, the stronger differences are only evident by improved resolution of triglycerides with the same number of carbon atoms: some peaks increase, others decrease as can be seen at the 44 and 46 peaks of the fractionated margarine fat in Figure 3. The molecular weight distribution would not reflect these changes at all. The chromatograms show that the late peaks of each group beyond triglyceride 40 are enriched in the crystallized fraction, reflecting the high tendency of the saturated molecules to solidify. Further identification of different peaks would

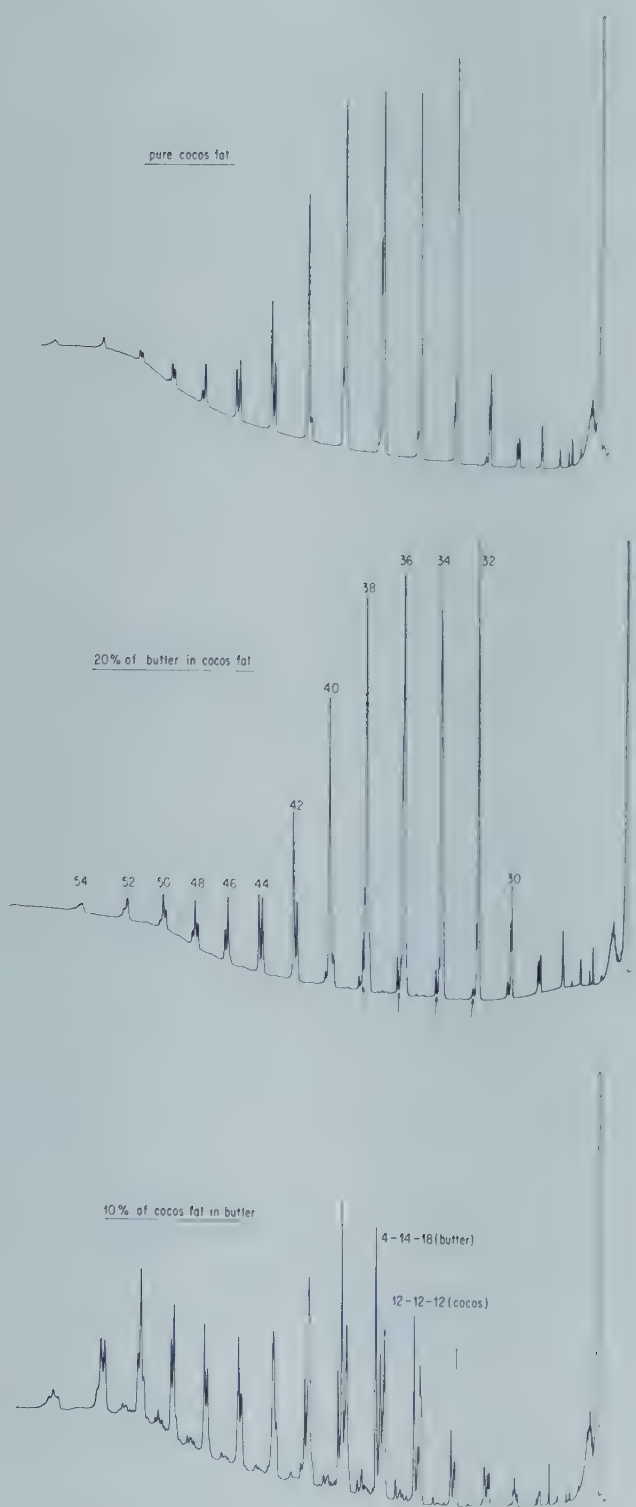

FIG. 2. Determination of butter fat/coconut oil mixtures by their triglycerides; limitations toward extreme compositions. Coconut oil (10%) can be easily determined in butter, but 20% of butter in coconut oil causes problems. A convenient quantitation can be achieved using an internal standard. For column and conditions, see Fig. 1.

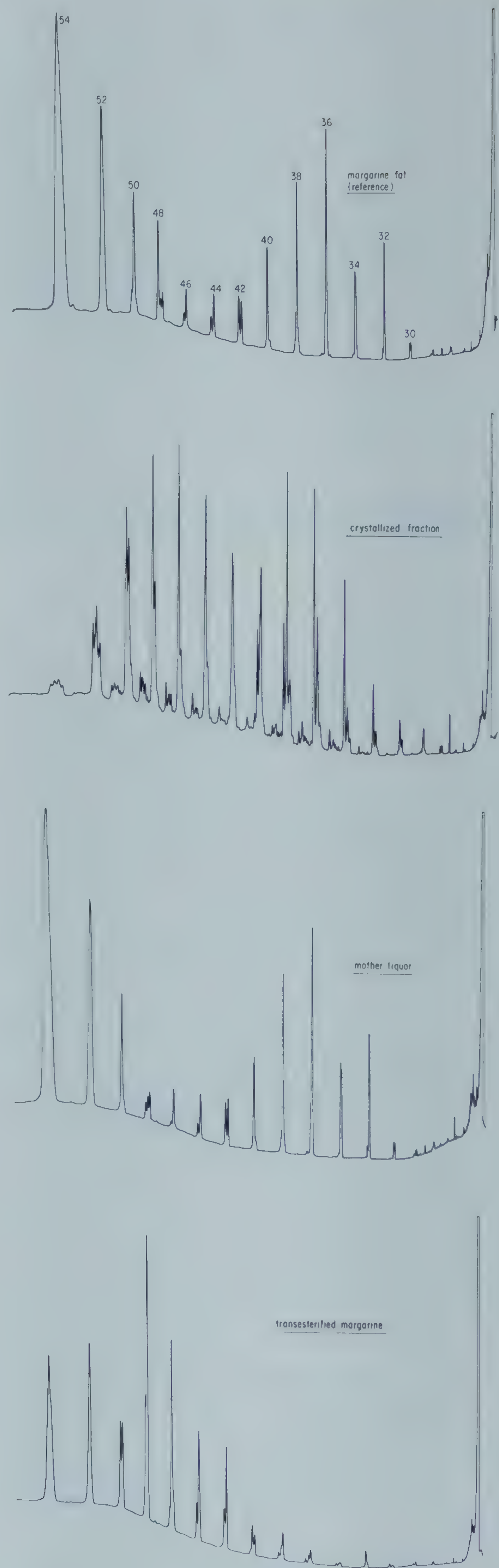

FIG. 3. Chromatogram of a commercial margarine, containing hydrogenated soybean oil and coconut oil. This margarine was fractionated and transesterified. Later peaks of each triglyceride group (probably representing the more saturated molecules) are concentrated into the crystalline fraction. Column and conditions as in Fig. 1.

probably facilitate the monitoring of fractionation processes.

Transesterification. Transesterification can be monitored by packed column GC, as shown by, e.g., Eckert (6). However, increased resolution as obtained with capillary columns gives more detailed information of the changes during such reactions. The butter and the margarine shown in Figures 1 and 3 may serve as examples. While the molecular weight distribution is strongly changed in the margarine, corresponding shifts are moderate only for the butter fat. Moreover a closer look at the fine structure within each group reveals dramatic changes (see groups 32-40 of the butter fat in Fig. 1). Again, much identification would be necessary to fully exploit the available information.

Heat-treated fats. Polyunsaturated fatty acids tend to polymerize when fats or oils are submitted to strong heat treatment. This process can be quantitated by the reduction of the fatty acid content using FAME analyses. The triglyceride analysis yields a complementary result as it reflects changes in the whole triglyceride molecule. Apart from the absolute reduction of triglyceride concentration there is usually a distinct change in their composition. Figure 4 shows the triglyceride chromatograms of a frying oil based on beef tallow, before and after heating. The late peaks of each group are increased because these largely saturated triglycerides show little tendency to polymerize.

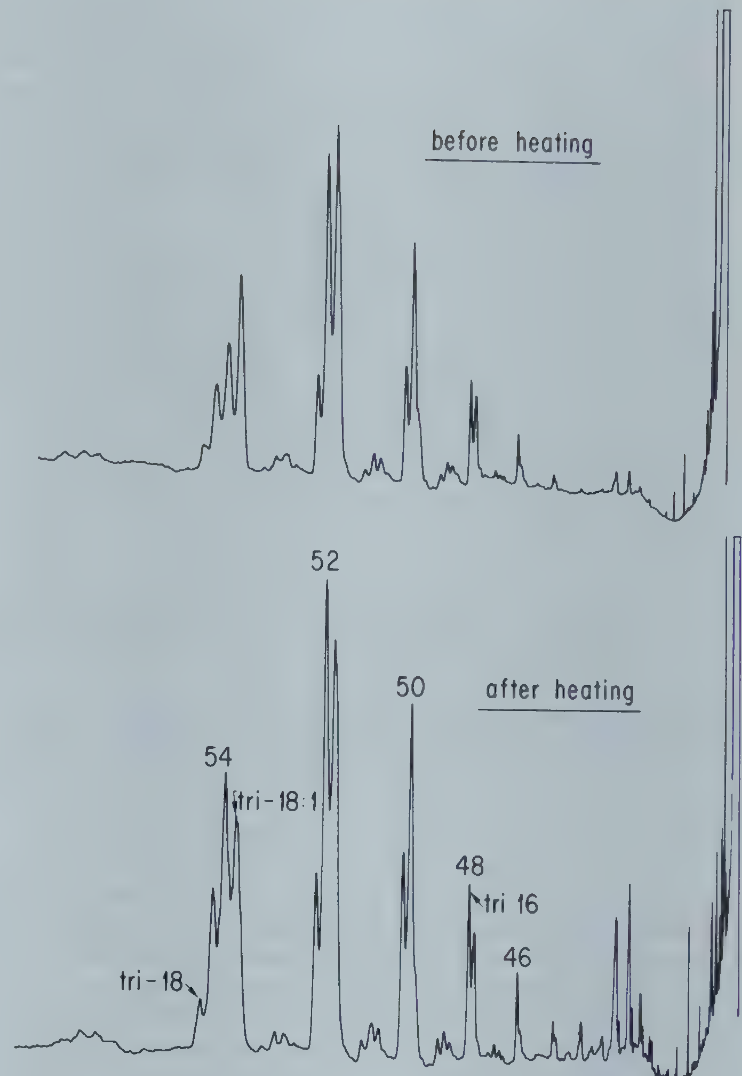

FIG. 4. Frying oils on the basis of beef tallow before and after heat-treatment. Positions of tri-16, tri-18 and tri-18:1 are marked. Besides a reduction of the total of original triglycerides, there is a relative change in peak size within each group of peaks, increasing the proportion of the saturated (later) peaks of a group at the cost of the unsaturated species (earlier peaks).

Remarks on the Technique

Columns for triglycerides. To be suitable for triglyceride analysis, capillary columns should fulfill 2 requirements: (a) they have to be thermostable up to at least 330 C. (350 C is preferable). This includes a support surface with sufficient inertness to avoid catalytic degradation of the stationary phase (causing high column bleeding and short column lives). Furthermore, the deactivation of the support has to be sufficiently thermostable to avoid tailing of the triglyceride peaks after extended use of the column.

(b) At temperatures above 300 C triglycerides are fairly labile compounds. While FAME at 150 C are only saponified on surfaces which completely absorb weak acids (as the test compound 2,6-dimethylphenol [15]), triglycerides decompose at 330 C on columns which elute weak acids almost perfectly. Satisfactory results were not obtained from those columns with basic bare glass surfaces beneath the stationary phase nor from columns prepared according to the "barium carbonate" method (16). In agreement with Schulte (9), "barium carbonate" columns had relatively short lifetimes and some of them even partially degraded triglycerides. However, persilanized columns (17,18) gave good results, even if programmed up to 360 C. These columns lasted for many runs without a notable change in the test chromatogram (checking adsorption, separation efficiency and retention according to Grob et al. [15]). Because of the low catalytic activity, a low bleed rate of such columns is achieved and degradation of triglycerides did not cause problems. As Grob describes (19), there was some discrimination of the biggest triglycerides, with losses ranging from 3 to 15%. This was assumed to result from degradation of these compounds at high temperature. However, this only brought the response down near 1 if normalized on the lower boiling triglycerides as tri-10 (instead of being ca. 9% higher for the tri-18 compared to tri-10).

Regarding selectivity for triglycerides, there was little difference between the gum phases such as OV 1, SE 30, 52 or 54. The unsubstituted methylpolysiloxanes seemed to be slightly preferable because of a somewhat lower elution temperature at comparable conditions (ca. 6 C lower for OV 1 compared to SE 52). Furthermore, unsaturated compounds are more clearly eluting before the saturated analog on the apolar phases. Film thickness varied between 0.08 and 0.12 μm as a compromise between a sufficient capacity (overriding baseline drift and ghost peaks) and low retention. The low retention reduces the thermal stress on the column and degradation of triglycerides.

Injection technique. The results of our studies comparing precision and accuracy of different injections for triglycerides (split, splitless and cold on-column) have been published elsewhere (19). They can be summarized as follows: split injections produced results with strong discrimination and very high standard deviations. Splitless injections gave results reflecting a discrimination resulting from insufficient elution out of the syringe needle. Since the different triglycerides eluted in similar proportions, discrimination seemed to be tolerable for many applications as long as a triglyceride was chosen as an internal standard. However, standard deviations were still on the order of 10%. Cold on-column injection had little discrimination and standard deviations of normalized results on an internal standard were 1-3%. Optimal conditions for cold on-column injections are described in (20). According to Monseigny et al. (10), the moving needle (or solid) injection is a viable alternative with little discrimination, and standard deviation is reported to be between 2 and 4%.

REFERENCES

1. Jaeger, H., H.H. Klör, G. Blos and H. Ditschuneit, in "Glass Capillary Chromatography," edited by R.E. Kaiser, Symposium Hindelang, Bad Dürkheim, 1975.
2. Grob, K., Jr., H.P. Neukom, D. Fröhlich and R. Battaglia, J. High Res. Chromatogr. CC 1:94 (1978).
3. Battaglia, R. and D. Frölich, Chromatographia (in press). to be published in Chromatographia).
4. Huebner, V.R., JAOCS 38:628 (1961).
5. Litchfield, C., Analysis of Triglycerides, Academic Press, New York, 1972.
6. Eckert, W.R., Fette Seifen Anstrichm. 79:360 (1977).
7. Takagi, T., and Y. Habashi, Lipids 12:1062 (1977).
8. Schomburg, G., R. Dielmann, H. Husmann and F. Weeke, J. Chromatogr. 122:55 (1976).
9. Schulte, E., 12th International Symposium on Chromatography, Baden-Baden 1978.
10. Monseigny, A., P.Y. Vigneron, M. Levacq and F. Zwoboda, Rev. Fr. Corps Gras 3:107 (1979).
11. Bezard, J., M. Bugant and G. Clement, JAOCS 48:123 (1971).
12. Beckenridge, W.E., and A. Kuksis, Lipids 3:291 (1968).
13. Grob, K., Jr., J. High Res. Chromatogr. (in press).
14. Eckert, W.R., Fette Seifen Anstrichm. 75:150 (1973).
15. Grob, K., Jr., G. Grob and K. Grob, J. Chromatogr. 156:1 (1978).
16. Grob, K., Jr., G. Grob and K. Grob, J. High Res. Chromatogr. CC 1:149 (1978).
17. Grob, K., G. Grob and K. Grob, Jr., Ibid. 2:31 (1979).
18. Grob, K., G. Grob and K. Grob, Jr., Ibid. 2:677 (1979).
19. Grob, K., Jr., Ibid. 178:387 (1979).
20. Grob, K., Jr. and H.P. Neukom, Ibid. 189:109 (1980).

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The Determination of Cocoa Butter Equivalents in Chocolate

F.B. PADLEY and **R.E. TIMMS**[1], Unilever Research Laboratory, Colworth House, Sharnbrook, Bedfordshire, England

ABSTRACT

A method of determining cocoa butter equivalents in chocolate and cocoa butter is described. The method relies on a new approach for interpreting data obtained by triglyceride gas liquid chromatography (GLC). This technique provides information on the composition of a fat according to the carbon number of the triglycerides (C_n). Examination of the data for a wide range of cocoa butters shows that a straight line relationship between the C_{50} and C_{54} contents exists. This relationship has been used as the basis for a quantitative method determining the amount and type of cocoa butter equivalent added to chocolate. The application of the method to both plain and milk chocolate is described. The method is also used to determine the amount of milk fat in chocolate.

INTRODUCTION

Throughout the world there is an increasing interest in the use of fats to partially replace cocoa butter in chocolate. The reasons, partly economic, are that certain fats can also provide significant product improvement (1). In the United Kingdom, Denmark and Ireland the addition of 5% non-cocoa butter fats, apart from milk fat, has been permitted for many years.

The type of fats that have been used in chocolate are very similar in chemical composition to cocoa butter. These fats mainly contain symmetrical triglycerides and for this reason it has been impossible to determine the amount of symmetrical-based fats in mixtures with cocoa butter.

Although there has been no method of monitoring the level to which added fats are used, the manufacturers have mutually agreed to abide by this 5% restriction. However it is desirable that some analytical method be made available which would quantitatively determine the amount of added fat in chocolate. The objective of the work described in this paper was to develop a method which would detect and quantify symmetrical triglyceride cocoa butter equivalents (CBE) when used at the 5% level in chocolate. Ideally, the method should also be quantitative at even lower levels of addition. The method should be suitable for both milk and plain chocolate, as well as rapid and easy enough for routine use.

The method is required to detect the high quality symmetrical-type CBE which have physical and chemical properties similar to those of cocoa butter. These CBE include Coberine, Illexao, illipe, shea fractions and palm fractions. Ideally, the method should also detect hardened fats but other methods are presently available for their characterization.

The determination of added fats has been the subject of much research and the position was reviewed in 1959 (2). At that time, modern chromatographic methods were being applied to oils and fats for the first time and this earlier work is of little interest today. Chromatographic methods for the general characterization of mixtures of oils and fats have been reviewed by Mani (3) and more recently for confectionery fats by Fincke (4).

Early attempts to detect added fats depended on the determination of the fatty acid composition of cocoa butter and, in particular, the content of lauric acid (5). Bonar (6) was able to detect 5% Coberine in cocoa butter using a preliminary low temperature crystallization as developed by Purr and Hettich (7,8). Methods dependent on lauric acid are unsuitable when milk fat is present. Iverson and Harrill (9,10) isolated the minor acids in cocoa butter, shea and illipe by urea fractionation. Although there was a suggestion that the method could be used to detect oils in cocoa butter, most operators would find the method difficult. The use of fatty acid analysis as a means of detecting added fats is therefore severely limited because some of them have a fatty acid composition similar to cocoa butter. Large additions of, e.g., palm fraction must be added before this method will detect it with certainty.

Sterol analysis is often used for identifying specific oils and fats. Fincke (11) has reported sterol analyses for several samples of illipe, Calvetta, Coberine and cocoa butter. Bracco et al. (12) have used sterol analyses to help characterize illipe/cocoa butter mixtures. Sterol analyses do have value in specific instances, e.g., detection of shea nut oil and its fractions or rapeseed oil. They are limited for detecting replacer fats because of their inability to detect palm fractions, major components of many products.

Sterol analysis is further limited because a preliminary step to isolate sterols is necessary and this is tedious. Quantitation is also virtually impossible because of the wide

[1] Present address: C.S.I.R.O., PO Box 20., Highett, Victoria, Australia.

variation in sterol content caused either by natural factors or processing.

The analysis of triglycerides using silver nitrate thin layer chromatography ($AgNO_3$-TLC) should allow cocoa butter replacers to be detected. In most cases a complete triglyceride analysis would be necessary and this requires too much skilled effort and time for routine use. The method could be of great value in isolating minor glyceride components unique to certain oils, e.g., the SSU (S = saturated; U = unsaturated) fraction of lard which could then be further characterized by triglyceride gas liquid chromatography (GLC).

The addition of marker compounds also has been suggested. These include rapeseed oil, BHT, sesamol, stigmasterol and, more recently, trinonanoin. A related triglyceride triheptanoin is already used to mark Intervention Butter in the EEC (13). Marker compounds have a number of disadvantages, plus, some manufacturers could leave them out of their products.

Confectionery fats also have been characterized by high temperature GLC. The general technique has been widely documented but the reader is directed particularly to the publications by Kuksis and Breckenbridge (14) and Litchfield (15). In addition, a number of workers have used the technique to characterized confectionery fats. Luddy et al. (15) characterized fractions of tallow and this helped optimize their process. The method has also been used to characterize mixtures of fats. For example, Warmbier (17) determined the amount of lard in goose drippings using a combination of $AgNO_3$-TLC and GLC. Rapeseed, soybean and rice bran oils were detectable in cottonseed oil at the 5-10% level (18). von Eckert (19,20) has also described various applications of triglyceride GLC.

Bracco et al. (12) used triglyceride GLC to detect illipe in cocoa butter. However, in all of these applications the quantitative estimation of added fats was inaccurate because of the natural variation in the composition of the fats.

In recent work (21), we found that triglyceride GLC could be usefully applied to confectionery fats. We described the reproducibility of the technique and typical correction factors for different triglycerides when operating under conditions similar to those described by Kuksis and Breckenbridge. In a separate brief communication (22), we have described a method of determining CBE in chocolate using triglyceride GLC data. We are aware that Fincke has independently developed a similar method which will be published shortly (personal communication).

In this paper we detail the method we have developed which overcomes inaccuracies from the natural variability of cocoa butter and allows the operator to determine quantitatively the amount of CBE added to chocolate or cocoa butter.

EXPERIMENTAL

In our early work we used a dual column technique, but if the column is properly conditioned a single column is satisfactory and gives a good baseline.

Instruments

A Pye 104 Model 64 Chromatograph equipped with a linear temperature programmer was used. Electronic integration was by a Chromalog 2 integrator (Kent Instruments).

Column

A glass, 2 foot × one-fourth inch od column was used which was fitted with graded glass-to-metal seals (Jencons, England).

Packing

Packing was 3% OV1 on 100-120 Gas-Chrom Q (Phase Separations).

Conditioning

Conditioning was basically as described by Kuksis and Breckenbridge (13). Our columns require at least 6 hr at 350 C.

Operating Conditions

A 7-cm injection needle was used so that its tip just reached into the column packing at the hottest part of the injection heater. Flow rate: 80 ml/min nitrogen; injection heater: 310-360 C, depending on the oven temperature (250-300 C), 360 C preferred injector temperature; detector heater: 380 C; programming rate: normally 4 C/min; sample: 1-2 μl of a 10% solution in chloroform.

Under these conditions, a confectionery fat containing triglyceride carbon numbers 46-56 can be analyzed in 20 min (300-355 C at 4 C/min plus 5 min hold at 355 C). Allowing ca. 10 min for the oven to cool back to 300 C, we found that 2 samples can be analyzed per hr.

The confectionery fats were not pretreated (i.e., no partial glycerides or free fatty acids, e.g., were removed) before analysis.

To obtain the highest precision and reproducibility we standardized our results using a standard cocoa butter which had been standardized by reference to a mixture of pure triglycerides. If analyses within a laboratory are self-consistent by reference to a standard cocoa butter, it is not necessary that the cocoa butter be absolutely standardized by reference to pure triglycerides. For milk chocolate analyses, a standard milk fat/cocoa butter mixture is used to insure high reproducibility. Products were sampled as described in the following sections.

Sample As Chocolate

When the sample is in the form of a chocolate bar or couverture the sample for analysis is prepared as follows: weigh out ca. 150 mg of the chocolate into a small phial and add 1 ml of analytical grade chloroform. Heat to 60 C, shake and keep at 60 C for ca. 5 min. Cool and centrifuge the solids to leave a clear or almost clear solution. Inject 2 μl of the supernatant directly into the GLC column.

We normally do not sample from the outer edges of a chocolate bar which might be contaminated on the surface or nonuniform, although our experience is that the fat phase of a chocolate bar is uniform. Duplicate samples are usually taken from different parts of the bar.

Some chocolate products such as vermicelli contain less fat than couvertures or bars. The vol of solution injected into the GLC column should be increased accordingly so that ca. 100 μg of fat is analyzed.

Sample As Fat

When the sample is available as fat, ca. 50 mg should be dissolved in 1 ml of analytical grade chloroform and 2 μl of the solution injected into the GLC column.

If a sample of cocoa butter is being analyzed, it may be in the form of a large block. Kleinert has reported nonuniformity in commercial cocoa butter blocks using the Pichard cooling curve test on samples from various parts of the block. To check this important point, we took samples from a 12-kg commercial block of cocoa butter and analyzed them in the usual way. The results in Table I show that there was no significant variation over the block.

TABLE I

Analyses of Samples from Various Parts of a Commercial Block of Cocoa Butter

Sample location	Carbon number (wt %)				
	48	50	52	54	56
Middle	0.2	16.9	47.1	34.7	1.1
Corner 1	0.2	16.8	47.3	34.6	1.1
Corner 2	0.3	16.3	47.2	35.1	1.1
Corner 3	0.2	16.7	47.2	34.8	1.1
Corner 4	0.1	16.9	47.2	34.7	1.1

NATURAL VARIABILITY OF COCOA BUTTER

Although much work has been reported on the variability of the chemical composition of cocoa butter, the majority of reported analyses refer to fatty acid methyl ester (FAME) analysis. There is little information available on the variability of the triglyceride composition.

The most comprehensive study of the variability of cocoa butter (by FAME analysis) has been reported by Woidich et al. (23,24). They analyzed 90 samples of cocoa butter which they extracted from beans of well defined origin. Large variations in the palmitic, stearic and oleic acid contents were observed for cocoa butters from different regions and from different harvests. These results were used as a starting point for our investigation of the variability of cocoa butter because we could not easily and quickly obtain such a wide variety of authentic cocoa butters.

To calculate triglyceride compositions from the FAME data we used the 1,3-random/2-random hypothesis of Van der Wal and Colemen and Fulton, which has been amply confirmed for cocoa butter. The FAME analyses at the 2-position were estimated by a method similar to that described by Litchfield (15). The method was applied to cocoa butters of known triglyceride analysis and shown to give accurate results.

Using this method, triglyceride compositions by carbon number and hence P_{50}, P_{52} and P_{54} values could be determined for a wide range of cocoa butters:

$$P_{50} = \frac{\text{\% of carbon no. 50}}{\text{\% of carbon nos. 50 + 52 + 54}} \times 100\%$$

i.e., the data for the 3 major peaks, 50, 52 and 54, is normalized so that $P_{50} + P_{52} + P_{54} = 100$. Examination of the data showed that P_{52} was practically constant for wide variations of P_{50} and P_{54}, and there was a linear relationship between P_{50} and P_{54}.

Following this encouraging result, we determined directly the triglyceride analyses by carbon number of a range of commercial cocoa butters. The Woidich et al. data is in many ways too comprehensive for our purpose as commercial samples of cocoa butter are not observed with such a wide variation in FAME analysis. This presumably is because the beans are blended together to give more uniform and consistent properties. We obtained 39 samples of cocoa butter or beans from chocolate manufacturers and cocoa butter producers in Great Britain and the Netherlands. The samples were chosen to be as near as possible to a random sample of the cocoa butters or beans which were available commercially in the years 1971-1974. The number of samples from each producing country was in approximate proportion to that country's production.

The 39 analyses were fitted to a straight line by the conventional least squares method giving:

$$P_{50} = 43.798 - 0.737^1 \ P_{54}, \qquad [I]$$

with a residual standard deviation of 0.1275.

The fit of the data to the line is shown in Figure 1. The equation of this line is similar to the line previously determined from our calculations using the Woidich et al. results. Workers in other laboratories could determine a slightly different line depending on how close their results are to absolute triglyceride percentages. Providing all the data in a given laboratory is referred to the same standard cocoa butter as already explained, this is unimportant since the results will be internally self-consistent. The important point is that the variability of the triglyceride composition of cocoa butter can be expressed by an equation of the form:

$$P_{50} = a - b \ P_{54},$$

where a and b are constants. We conclude that all analyses of pure cocoa butter from whatever source must lie on this line. We can also describe the distribution of the cocoa butter analyses by the means and standard deviations of P_{50} and P_{54}:

mean P_{54} = 34.752 $\quad \sigma_{54} = 1.022$
mean P_{50} = 18.183 $\quad \sigma_{50} = 0.764$

These parameters can then be used to describe the distribution of the analyses along the line if we assume the natural variability of cocoa butter analyses to be normally distributed. Figure 2 illustrates the distribution of the P_{54} values with a histogram. The normal distribution

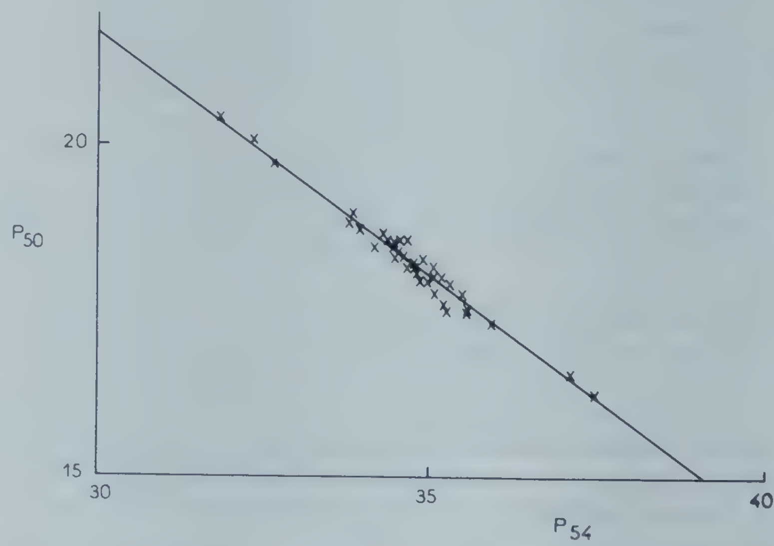

FIG. 1. The natural variability of cocoa butter: linear relationship between P_{50} and P_{54}.

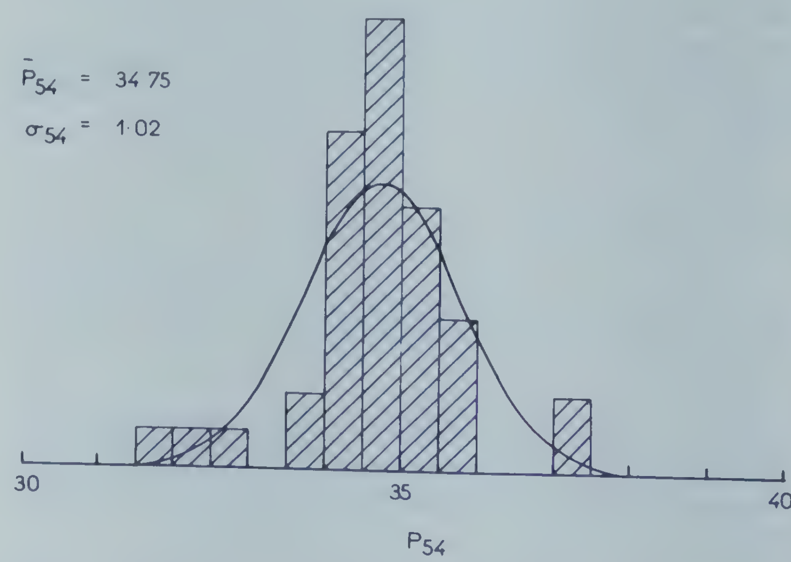

FIG. 2. The natural variability of cocoa butter: comparison of histogram distribution with normal distribution.

curve deduced from the mean and standard deviation just given is superimposed for comparison. In view of the limited amount of data, there is a reasonable approximation to a normal distribution. All the data lies within the limits which would contain 99.8% of the normal distribution. Eighty-eight percent of the data lies within the limits which would contain 95% of the normal distribution. The extreme analyses do seem to be associated with the smaller producing regions. For example, the highest P_{54} value comes from Sabah (Malaysia) and the lowest from Grenada (West Indies). Subsequent analyses of more than 40 cocoa butters extracted from commercial chocolates have all been within the 99.8% limits with mean and standard deviation similar to those already given.

INTERPRETATION OF GLC DATA

All CBE added to cocoa butter will cause the triglyceride analysis to deviate from the line described by Equation I to the extent that the P_{52} value of the CBE is different from the P_{52} value of cocoa butter. All possible commercial CBE made solely from vegetable fats contain fewer carbon-number-52 triglycerides than are found in cocoa butter. Hence all CBE will cause deviations from Equation I and will be detected (Fig. 3). Unlike FAME analyses, where the deviation resulting from a fat rich in palmitic acid can be cancelled out by adding a fat rich in stearic acid, deviations from Equation I cannot be cancelled out by adding a second CBE of different triglyceride analysis. Only lard and tallow and their fractions have high contents of carbon-number-52 triglycerides such that in mixtures with vegetable-based CBE they might escape detection by our method. Animal fats usually can be detected by sterol analyses or by $AgNO_3$-TLC. Typical analyses for several CBE are given in Table II.

It is possible to quantify the deviation from the line and thus determine the percentage and type of CBE added. Consider a CBE, A, with analysis $P_{50}{}^{A}$, $P_{52}{}^{A}$, $P_{54}{}^{A}$. A mixture of A and cocoa butter is made to contain a fraction x (by weight) of A. By mass balance (neglecting triglycerides with carbon numbers other than 50, 52 and 54 as negligible, which is discussed in more detail in Discussion, we have:

$$P_{50}{}^{CB+A} = P_{50}{}^{CB}(1-x) + P_{50}{}^{A}\cdot x$$
$$P_{54}{}^{CB+A} = P_{54}{}^{CB}(1-x) + P_{54}{}^{A}\cdot x \qquad \text{[II]}$$

where $P_{50}{}^{CB+A}$, $P_{54}{}^{CB+A}$ and $P_{50}{}^{CB}$, $P_{54}{}^{CB}$ are the analyses of the mixture and the cocoa butter, respectively.

Solving Equations II and III with Equation I we have:

$$P_{50}{}^{CB+A} = 43.798(1-x) + x(P_{50}{}^{A} + 0.737^{1}\ P_{54}{}^{A}) - 0.737^{1}\ P_{54}{}^{CB+A} \qquad \text{[IV]}$$

Equation IV is the equation of a family of straight lines parallel to the line described by Equation I.

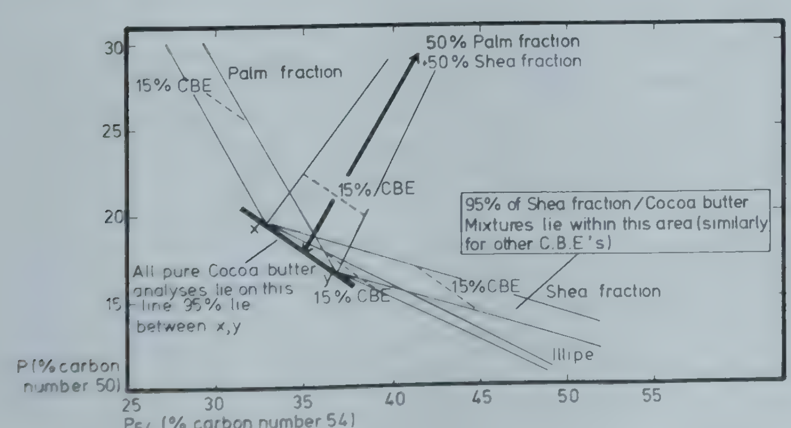

FIG. 3. Effect of adding CBE to cocoa butter.

TABLE II

The Composition of Different CBE

CBE	No. of samples	Average P_{50}	Average P_{54}
Calvetta	17	75.52	3.04
Shea fraction	14	0.76	90.51
Coberine	21	38.22	34.37
Choclin	21	49.65	28.72
Illipe	22	7.24	54.84
Shea oil	8	0.81	89.27
Sal oil	5	1.45	81.80
Mowrah oil	1	19.10	34.95
Kokum oil	1	0.57	93.75
Illexao 30-61	1	38.0	38.0
Illexao 30-96	2	21.30	64.77

Compositions must be determined periodically because of variation in composition.

To determine the locus of a given point $P_{50}{}^{CB+A}$, $P_{54}{}^{CB+A}$ as A is added, i.e., the line connecting $P_{50}{}^{CB}$, $P_{54}{}^{CB}$ and $P_{50}{}^{A}$, $P_{54}{}^{A}$ we eliminate x between Equations II and III to give:

$$P_{50}{}^{CB+A} = \frac{P_{54}{}^{CB+A}\cdot(P_{50}{}^{A} - P_{50}{}^{CB})}{(P_{54}{}^{A} - P_{54}{}^{CB})} + \frac{P_{50}{}^{CB}\cdot P_{54}{}^{A} - P_{54}{}^{CB}\cdot P_{50}{}^{A}}{P_{54}{}^{A} - P_{54}{}^{CB}} \qquad \text{[V]}$$

Using Equations IV and V and given any CBE whose analysis is known, a family of lines can be drawn rapidly showing the effect of adding various percentages of CBE to any sample of pure cocoa butter. In practice, CBE are variable in analysis because of blending, processing or natural variability. It is possible to express this variability statistically, leading to a modification to Equation V.

Quantitative Estimation with the Aid of Diagrams

To illustrate the use of Equations IV and V, Figure 4 shows the data calculated for the 3 important CBE or CBE components; palm fraction, shea fraction, illipe (Borneo Tal-

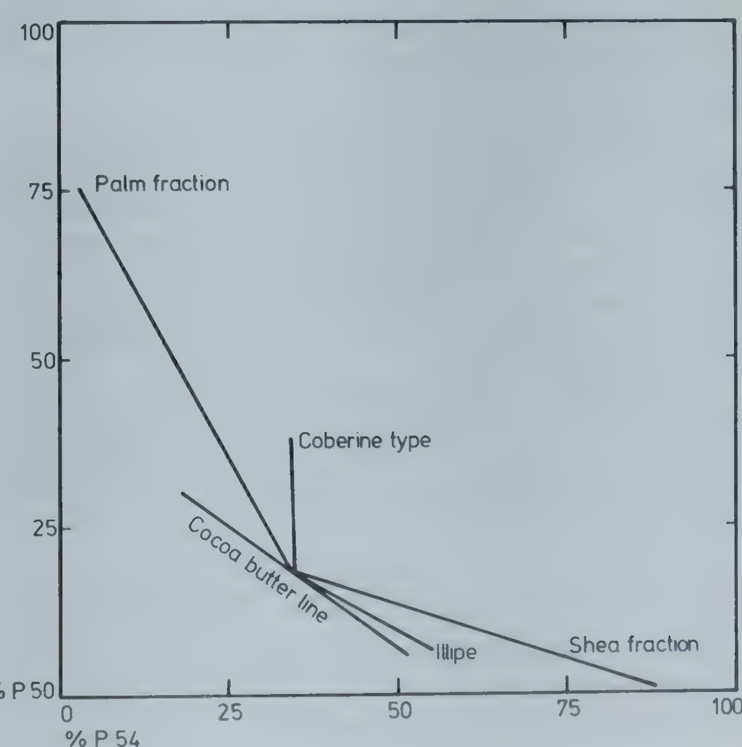

FIG. 4. Detection of CBE by triglyceride GLC method.

low), together with the data for a typical CBE consisting of a mixture of palm fraction and shea fraction. The lines show the limits within which 95% of all CB/CBE mixtures lie assuming the variability of the CBE is as we have determined by sampling from Unilever factories. Similar diagrams can be drawn for any other CBE of interest. To use the diagrams, the analysis is plotted on transparent graph paper and placed over each diagram in turn.

If the analysis lies on the cocoa butter line (Equation I), it is deemed to be pure cocoa butter. If it deviates from the line (for vegetable fats it can do so in an upward direction only) then each CB/CBE diagram is checked to see within which CB/CBE field it lies. Finally, the CBE percentage is read; for clarity only the 15% lines are shown in Figure 4. Sometimes, especially for low additions of CBE, it will not be possible to distinguish unequivocally between similar CBE. In such cases, alternative results must be quoted. Additional evidence will then be required if a firmer conclusion is necessary.

To provide more information than is easily obtained using the graphical method of interpretation just described, we have developed a rigorous mathematical interpretation of the data using standard statistical methods. Using a computer, the data may be rapidly processed and results given including confidence limits. All the results given in the following section have been obtained this way. The method of calculating 95% confidence limits and of deciding whether an analysis lies on the cocoa butter line is given in the following section.

Deciding if a Sample Is Pure Cocoa Butter

Pure cocoa butter is assumed to be defined by Equation I. If the data is normally distributed, then 99% of the analyses are beneath the average value plus 2.326 x residual standard deviation. Therefore, pure cocoa butter should comply with:

$$P_{50} < 43.798 - 0.737^1\ P_{54} + 2.326 \times 0.1275,$$
$$\text{i.e., } P_{50} < 44.095 - 0.737^1\ P_{54},$$

for 99% of all analyses. Greater values of P_{50} are taken to mean that the sample is not pure cocoa butter.

Calculation of % CBE in a CB/CBE Mixture

If we solve Equation VI for x we get:

$$x = \frac{c}{d} = \frac{P_{50}{}^{CB+A} - 43.798 + 0.737^1\ P_{54}{}^{CB+A}}{P_{50}{}^{A} - 43.798 + 0.737^1\ P_{54}{}^{A}}, \qquad \text{[VI]}$$

where x is the fraction of CB/CBE mixture.

In the derivation of Equation IV it was assumed that components other than triglycerides with carbon numbers 50, 52 and 54 were negligible and could be neglected. This is a reasonable assumption for cocoa butter and many CBE but for certain oils, other glycerides or unsaponifiable matter must be considered. One such oil is sal oil (*Shorea robusta* seed oil), which contains 15-20% of triglycerides with carbon number 56.

To calculate x more rigorously we define f^{CB}, f^{A} or f^{CB+A} as the fraction of the CB, CBE or mixture which consists of triglycerides with carbon numbers 50, 52 and 54. Then, instead of our earlier Equations II and III we have:

$$P_{50}{}^{CB+A} \cdot f^{CB+A} = P_{50}{}^{CB} \cdot f^{CB}(1-x) + P_{50}{}^{A} \cdot f^{A} \cdot x \qquad \text{[VII]}$$
$$P_{54}{}^{CB+A} \cdot f^{CB+A} = P_{54}{}^{CB} \cdot f^{CB}(1-x) + P_{54}{}^{A} \cdot f^{A} \cdot x \qquad \text{[VIII]}$$

Summing Equations VII and VIII and the equivalent equation for P_{52} we deduce:

$$f^{CB+A} = (1-x)f^{CB} + xf^{A}, \qquad \text{[IX]}$$

since $P_{50} + P_{52} + P_{54} = 100$ by definition.

Combining Equations VII, IX and I, we deduce that:

$$x = \frac{x'}{f^A/f^{CB} + (1-f^A)x'}, \qquad \text{[X]}$$

where x' is the value of x deduced from the approximate Equation VI and x is the exact value. To estimate f, one has to take into account triglycerides other than 50, 52 and 54, partial glycerides and unsaponifiable matter. Our experience is that the correction implied by Equation X is negligible for most CBE except for sal oil (mentioned previously) or unfractionated shea oil which is rich in unsaponifiables and partial glycerides.

Accuracy of Estimated % CBE

If we assume that all variations are normally distributed and that the unknown sample of chocolate has been mixed with a randomly chosen batch of CBE, then we can calculate confidence limits for % CBE using standard statistical methods.

Although to be exact one should apply Equation X after Equation VI, x and x' are so similar that for the estimation of the confidence limits we can just use Equation VI.

From Equation VI, since we do not know the values of P_{50} and P_{54} for the particular CBE which has been used, the best estimate of x which we can make is: $x = \frac{c}{\mu_d}$, where $\mu_d = \overline{P}{}^A_{50} - 43.798 + 0.737^1\ \overline{P}{}^A_{54}$ and $\overline{P}{}^A_{50}$ and $\overline{P}{}^A_{54}$ are the mean values obtained from the analysis of a random selection of samples of the CBE.

To obtain confidence limits for x we need a function of x whose mean and standard deviation can be calculated. A suitable function is: $\theta = d.x - c$. If x is the true proportion of CBE, it follows from Equation VI that $\mu_\theta = 0$. For any determination, θ is estimated by $(\mu_d x - c)$ and will differ from 0 because d for the particular CBE will not equal μ_d and because c varies between determinations with a standard deviation which we can assume to be the variation about Equation I for cocoa butter. Hence:

$$\sigma_\theta{}^2 = \sigma_d{}^2 \cdot x^2 + \sigma_c{}^2,$$

where $\sigma_c = 0.1275$, $\sigma_d{}^2 = (\sigma_{50}{}^A)^2 + (\sigma.7371\ \sigma_{54}{}^a)^2 + 1.4742\ \sigma^A_{5054}$ and $\sigma_c{}^2$, $(\sigma_{50}{}^A)^2$, e.g., are the variances of c, percentage of carbon number 50 in A, e.g., respectively and σ^A_{5054} is the covariance of the percentages of carbon numbers 50 and 54 in A. $|\mu_d . x - c| < 1.96\ \sigma_\theta$ in 95% of determinations. It follows that confidence limits (probability = 0.95) are given by solutions of:

$$(\mu_d . x - c)^2 = 3.8416\,(\sigma_d{}^2 . x^2 + \sigma_c{}^2)$$

$\therefore$ 95% confidence limits =

$$\frac{\mu_d . c \pm \sqrt{\mu_d{}^2 . c^2 - (\mu_d{}^2 - 3.8416\,\sigma_d{}^2)(c^2 - 3.8416\,\sigma_c{}^2)}}{\mu_d{}^2 - 3.8416\,\sigma_d{}^2}$$

Milk Chocolate

When milk chocolate is analyzed it is necessary to correct the observed triglyceride analysis for the presence of milk fat triglyceride in the carbon number 50, 52 and 54 region. This correction is small and we use the sum of the analyses of the carbon-number-40-, 42- and 44-triglycerides both to estimate the amount of milk fat and to determine the necessary correction to the carbon-number-50, 52- and 54-triglycerides. The sum of triglycerides 40, 42 and 44 is used because it shows smaller variability with different milk fats than the single triglyceride 40 or a wider range of glycerides because it minimizes interference from the diglyceride or triglyceride peaks of the cocoa butter or CBE.

We have found that the total butter fat peaks between C_{26} and $C_{60} = (C_{40} + C_{42} + C_{44}) \times 4.270$ and this relation-

ship can be used to calculate the percentage butterfat, e.g.,

$$\% \text{ Butterfat} = \frac{(C_{40} + C_{42} + C_{44}) \times 4.270}{\sum_{20}^{60} C_i}$$

Each operator should, however, determine this factor using appropriate standard mixtures.

In order to correct the peaks at 50-54 resulting from the presence of butterfat we have used the following relationship: 10% (C_{40} + C_{42} + C_{44}) is equivalent to: C_{50} = 4.7; C_{52} = 4.7; and C_{54} = 2.6. Using this relationship, it is possible to correct the peaks in the 50-54 region before determining the level of CBE.

RESULTS

To confirm the accuracy of the method, several fat and chocolate samples of known composition were prepared using the CBE Calvetta. Various types of chocolate products were prepared including vermicelli and flake chocolate (Table III).

The agreement between observed and calculated analyses is good. Only one analysis lies outside the estimated 95% confidence limits. Vermicelli-type products are prepared by dry mixing or kneading without melting. The ingredients are thus not as well mixed as in normal chocolate and this may have caused the observed discrepancy.

Besides Calvetta, other CBE were possible as alternative interpretations of the data especially at low levels of adulteration. This is not usually a problem as at the 15% level of CBE in fat (∿5% on chocolate) which is of greatest interest, the CBE analyses overlap less or not at all. Other analyses such as sterol analysis can then be used if required to help to confirm a particular CBE.

In Table IV we give the results of the analysis of chocolates purchased in Great Britain and Canada. Results are given in terms of the main CBE sold for use at the 5% level in chocolate in Britain, e.g., coberine, illipe (Borneo Tallow), Illexao 30-96, Illexao 30-61. Calvetta is also included. Where "zero" is given, there is less than a 0.2% (1 in 500) chance of the product consisting of a mixture of cocoa butter and that particular CBE; where "low" is given, a less than 5% (1 in 20) chance.

The results in Table IV allow the percentage of CBE in the chocolate to be calculated in terms of commercial products when the fat content of the chocolate is known. The fat content can be determined by standard procedures, but for routine screening of products it is sufficient to assume that one-third fat is present.

In the United Kingdom the present legislation permits the addition of 5% vegetable fat CBE to chocolate. From the results it is clear that most manufacturers have been adding a CBE and that many are adding the full 5% permitted by law.

The limited results in Table IV on 3 products from 2 manufacturers do indicate that CBE are not present in Canadian chocolate, in marked contrast to the results for British chocolate.

DISCUSSION

The method of analysis and the interpretation of the results outlined in this paper have 3 main advantages over existing methods for the detection and determination of CBE in chocolate.

First, the method is almost foolproof because it depends on the analysis of triglycerides, the essential and major component of fats. Methods depending on the detection of minor components such as sterols can often give misleading or wrong results because such components may be removed without noticeably affecting the physical characteristics of the fat. Thus, solvent crystallization, bleaching and deodorization, processes which are all used in the production of CBE, can significantly reduce the level of minor components such as sterols. Methods depending only on the addition of marker compounds are inherently vulnerable to unscrupulous manufacturers who may not add the marker compound.

Second, the method is quantitative, and reliable confidence limits can be assigned to the results. The interpretation of the data provided by the method will depend in part on the additional information that can be provided—e.g., by the chocolate or CBE manufacturer. If no information is available about the sample then a list (normally limited to 2 or 3 possibilities) of possible CBE and the amounts present can be calculated (see Table IV). If the type of CBE is known, it will be estimated quantitatively and the other alternatives can be ignored.

Third, the method is rapid and capable of automation. Total time for sampling, analysis and interpretation of the results using a computer is no more than 45 min for milk or plain chocolate. The GLC analysis can easily be automated so that ca. 50 analyses can be made in 24 hr. Auto-

TABLE III

Comparison of Observed (from GLC Analysis) and Calculated (from Recipe) Results

Product type	% Milk fat in total fat	Pure cocoa butter present	% Calvetta in CB/CBE (i.e., excluding milk fat)		
			Found	Range given by 95% confidence limits	Calculated
Bar	-	No	1.8	1.0 - 2.6	2.1
Vermicelli	19.7 ± 2.6	No	10.8	9.8 - 11.8	13.3
Vermicelli	22.6 ± 3.1	No	11.8	10.8 - 12.8	12.6
Vermicelli	-	No	14.0	13.0 - 15.0	14.7
Vermicelli	-	Yes	-	-	0
Flake	13.2 ± 1.5	Yes	-	-	0
Flake	-	Yes	-	-	0
Bar	9.5 ± 1.7	Yes	-	-	0
Bar	-	Yes	-	-	0
Fat	-	No	6.4	5.4 - 7.4	6.0
Fat	-	No	7.5	6.5 - 8.5	7.0
Fat	-	No	5.8	4.8 - 6.8	5.0
Fat	-	Yes	-	-	0
Fat	-	Yes	-	-	0

mation should lead to even higher precision than we have achieved using manual injection with relatively unsophisticated GLC equipment available in most analytical laboratories.

Using the equations and method of calculation outlined in Interpretation of GLC Data, it is possible to give minimum detection limits and 95% confidence limits at the 15% CBE level for any CBE. Samples of the particular CBE must be analyzed to establish the variability of the CBE. The average results, calculated for 5 CBE, assuming each CBE is added separately to cocoa butter, are shown in Table V. The presence of milk fat has no significant effect on the minimum amount which can be detected or the confidence limits, when they are expressed as the % CBE in the CB/CBE mixture, excluding milk fat.

These figures clearly show the difficulty of detecting and quantifying illipe alone. This is because illipe has a triglyceride analysis similar to the cocoa butter analysis. The problem is clearly shown in Figure 3. In practice, as indicated in Table IV, it is often possible to show that illipe cannot be present on its own, so that the problem may not be very important, especially considering the relatively small quantity of illipe available.

Should it be possible to obtain a sample of the cocoa butter used to formulate the chocolate, the precision of detection and quantification of illipe and other fats can be much improved (see Table V). For instance, the minimum detection limit and the confidence limits can then be made comparable with the figures given for Coberine. *By analyzing several chocolates from a given chocolate manufacturer containing different levels of the same CBE it is possible to deduce the analysis of the cocoa butter used*, assuming it is the same for all the chocolates analyzed. This follows from equation V. The information is then available to improve the precision of the illipe analysis. The precision of detection of palm oil may be similarly improved. Alternatively, palm oil can be detected at a level of 1% or less if the triglyceride analysis is done on a trisaturated triglyceride fraction isolated by crystallization or $AgNO_3$-TLC. A similar procedure has been used to determine lard in goose drippings (17).

In some countries, poor quality solvent-extracted cocoa butter is available and may be added to chocolate. This poor quality cocoa butter contains cocoa shell fat. We have

TABLE IV

The Composition of the Fat Phase of Some British and Canadian Chocolate Products

Country	Type of product	Pure cocoa butter present	Milk fat[a] (%)	Alternative CBE possible[b] Calvetta (%)	Coberine (%)	Illipe (%)	Illexao 30-92 (%)	Illexao 30-96 (%)
UK	Milk chocolate bar	No	21.3 ± 2.3	zero	16.4 ± 2.8	zero	zero	12.9 ± 1.1
UK	Milk chocolate bar	No	20.8 ± 2.0	9.5 ± 0.9 low	17.0 ± 2.9	zero	zero	13.4 ± 1.1 low
UK	Milk chocolate bar	No	27.6 ± 3.5	11.0 ± 1.0	19.4 ± 3.1	zero	zero	zero
UK	Milk chocolate bar	No	29.9 ± 3.3	zero	24.6 ± 3.9	zero	zero	zero
UK	Chocolate-coated toffee bar	No	19.5 ± 2.0	zero	22.5 ± 3.5	zero	zero	17.6 ± 1.2
Canada	Molded chocolate shapes	Yes	3.3 ± 0.7	-	-	-	-	-
UK	Chocolate-coated peppermint cream	Yes	5.9 ± 0.9	-	-	-	-	-
UK	Chocolate-coated peppermint cream	No	-	5.3 ± 0.8 low	9.3 ± 1.9	zero	zero	7.3 ± 1.1 low
UK	Plain chocolate bar	No	14.3 ± 1.3	1.2 ± 0.8 low	2.1 ± 1.3	11.0 ± 8.0	1.7 ± 1.1	1.6 ± 1.0
UK	Chocolate-coated wafer	No	24.7 ± 2.4	zero	20.1 ± 3.3	zero	zero	15.7 ± 1.1 low
Canada	Chocolate-coated wafer	Yes	28.1 ± 3.2	-	-	-	-	-
Canada	Plain chocolate bar	Yes	9.8 ± 0.8	-	-	-	-	

[a]Percentage in total fat.

[b]Percentage in CB/CBE phase, excluding milk fat.

TABLE V

Effect on Confidence Limits if Either CB or CBE Is Known Precisely[a]: 95% Confidence Limits at 15% CBE Addition

CBE	Neither CB nor CBE known: Graph[b]	P_{50}	P_{54}	CB known, CBE unknown: Graph	P_{50}	P_{54}	CBE known, CB unknown: Graph	P_{50}	P_{54}	CB and CBE known: Graph	P_{50}	P_{54}
Calvetta	1.1		6.5	0.9		0.7	0.8		6.5	0.4		0.6
Illexao 30-92	1.1		3.7	0.7		0.6	1.1		3.7	0.5		0.4
Coberine	2.5		-[c]	2.4		-[c]	1.3		-[c]	0.6		48
Illipe	8.9		10.7	6.9	1.8	2.8	6.5	7.2	10.2	3.1	1.4	1.0
Illexao 30-96	1.1		17.2	0.8		1.9	1.0		17.1	0.5		1.5

[a]e.g., 15% Calvetta would be determined with 95% confidence to give a result 15 ± 1.1% using the line method and no prior knowledge.

[b]Method described in this paper.

[c]Effectively 100%.

analyzed several cocoa shell fats and poor quality cocoa butters. Such fats do not affect our method of analysis.

Nut oils from nuts added to chocolate will be detected and in most cases it is not possible to distinguish them from oils such as shea oil by this method. In this case slightly more sophisticated methods would have to be used if a correction for nut oils was required.

The accuracy and speed of this method for the detection and the determination of CBE in chocolate make it ideal for the routine analysis of chocolate for the purpose of minotoring the addition of CBE. In just a few cases, the method will not yield unequivocal results and supplementary analyses such as sterol or *trans* acid determination would be required.

ACKNOWLEDGMENT

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REFERENCES

1. Padley, F.B., C.N. Paulussen, C.J. Soeters and D. Tresser, Rev. Int. Choc. 27:226 (1972).
2. Purr, A., Fette Seifen Anstrichm. 61:675 (1959).
3. Mani, V.V.S., and G. Lakshminarayani, Chromatogr. Rev. 10:159 (1968).
4. Fincke, A., Dtsch. Lebensm. Rundsch. 71:284 (1975).
5. Purr, A., Rev. Int. Choc. 14:204 (1959).
6. Bonar, A.R., Ibid. 20:103 (1965).
7. Purr, A., and A. Hettich, Rev. Int. Choc. 15:548 (1960).
8. Purr, A., Ibid. 15:456 (1960).
9. Iverson, J.L., P.G. Harrill and R.W. Weik, J. Assoc. Off. Anal. Chem. 52:685 (1969).
10. Iverson, J.L., and P.G. Harrill, Ibid. 50:1335 (1967).
11. Fincke, A., Fette Seifen Anstrichm. 73:534 (1971).
12. Bracco, U., W. Rostagno and R.H. Egli, Rev. Int. Choc. 25:44 (1970).
13. Sadini, V., Riv. Ital. Sostanze Grasse 52:331 (1975).
14. Kuksis, A., and W.C. Breckenbridge, J. Lipid Res. 7:576 (1966).
15. Litchfield, C., in "Analysis of Triglycerides," Academic Press, 1972.
16. U.S. Patent 3,944,585.
17. Warmbier, M., Fette Seifen Anstrichm. 76:86 (1974).
18. Imai, C., H. Watanabe, N. Haga and T. Ii, JAOCS 51:326 (1974).
19. von Eckert, W.R., Fette Seifen Anstrichm. 75:150 (1975).
20. von Eckert, W.R., Ibid. 77:360 (1977).
21. Padley, F.B., and R.E. Timms, Lebensm. Wiss. Technol. 11:319 (1978).
22. Padley, F.B., and R.E. Timms, Chem. Ind. (London) 1978:918.
23. Woidich, H., H. Gnauer, Q. Reidl and G. Galinowsky, Lebensm. Untersuchung Forsch. 125:9 (1964).
24. Woidich, H., H. Gnauer, Q. Reidl and G. Galinowsky, Ibid. 143:104 (1970).

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Selective Hydrogenation with Copper Catalysts: V. Kinetics and Mechanism at High Pressure[1]

S. KORITALA, Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, IL 61604

ABSTRACT

The mechanism of hydrogenation at 900-950 psi with copper-chromite catalyst was investigated with pure methyl esters as well as their mixtures. A comparison of double bond distribution in *trans*-monoenes formed during hydrogenation of linoleate and alkali-conjugated linoleate revealed that 85-95% of the double bonds in linoleate conjugated prior to hydrogenation. The mode of hydrogen addition to conjugated triene and diene at high pressure is similar to that at low pressure but positional and geometric isomerizations of unreduced conjugated esters were less at high pressure. Geometric isomerization of methyl linoleate and linolenate was considerable at high pressure whereas it was negligible at low pressure. The absence of conjugated products during hydrogenation of polyunsaturated fatty acid esters resulted from their high reactivity. Conjugated dienes are 12 times more reactive than the triene, methyl linolenate, and 31 times more reactive than the diene, methyl linoleate. The products of methyl linolenate hydrogenation were the same as those predicted by the conjugation mechanism.

INTRODUCTION

Studies on the selective hydrogenation of soybean oil with copper catalysts at pressures between 500 and 3000 psi (1) revealed a number of features that are different from low-pressure hydrogenation. Minor amounts of conjugated dienes that appear in the product during low-pressure hydrogenation were eliminated at high pressures. The amount of *trans* isomers formed for each unit of iodine value drop was significantly greater at high pressures. Despite these differences, the selectivity for the reduction of linolenate remained the same. According to theory (2), increased pressure decreases linoleate selectivity (S_L) and *trans* isomerization. In the hope of explaining the anomalous behavior of copper catalysts at high pressures, the mechanism of hydrogenation was investigated with pure unsaturated fatty acid esters varying in number, position and geometric configuration of double bonds as model compounds. The relative reaction rates were determined by hydrogenating mixtures of fatty acid esters. The results of these studies were compared with those obtained at low pressure (3-5) where conjugation was shown to be an essential step prior to hydrogenation.

[1] Presented at the 70th Annual Meeting of the American Oil Chemists' Society, San Francisco, April 29-May 3, 1979.

EXPERIMENTAL PROCEDURES

Preparation of Pure Methyl Esters

Methyl linolenate (6) and methyl linoleate (7) were prepared by counter double-current distribution of linseed and safflower oil esters, respectively. β-Eleostearic ($t9,t11,t13$-octadecatrienoic) acid was prepared from tung oil by low-temperature crystallization (8) and esterified with methanolic hydrochloric acid and 2,2-dimethoxy propane (9). Methyl $c9,t11$- and $t10,c12$-octadecadienoate mixture was obtained by conjugation of methy linoleate with alkali.

Hydrogenation. Reductions were carried out in a 150-ml, magnetically stirred, Magna-Dash autoclave. The fatty ester and 0.5% commercial copper chromite catalyst (Harshaw-CU 1106P) were heated electrically to 170 C under vacuum.

With β-eleostearate reaction was carried out at 150 C. Hydrogen was admitted into the autoclave to 950 psi. When pressure dropped to 900 psi, hydrogen gas was readmitted to 950 psi. Samples were withdrawn at predetermined intervals.

Analytical methods. Fatty acid compositions of partially hydrogenated methyl esters were determined isothermally at 190 C with a Varian Aerograph Model 1860 GC instrument equipped with dual 6 ft × 1/8 in. stainless-steel columns, packed with 15% EGSS-X on Gas-Chrom P, 100/120 mesh and with flame ionization detectors. Nitrogen carrier gas was used at a flow rate of 20 ml/min. The percentage of each ester was calculated as the percentage of the total area of all the peaks. Conjugatable dienes were determined by the official AOCS method (10) except that alkali-conjugation was carried out for 1 hr. The percentage of isolated *trans* unsaturation was measured by infrared (IR) absorption of methyl esters at 10.36 μm and by comparison with methyl elaidate standard. Methyl esters were separated into saturates, monoenes, dienes and trienes by high performance liquid chromatography (HPLC) (11). Conjugated dienes and conjugated trienes could not be separated by HPLC. Therefore, hydrogenated product from β-eleostearate was separated by reverse-phase chromatography on a rubber column (12). Further separation of monoenes into *cis-* and *trans-*fractions and conjugated dienes into *cis,trans-* and *trans,trans-*fractions was accomplished on a silver-exchanged resin column (13). Positional isomers of various monoene, conjugated diene and conjugated triene fractions were determined by reductive ozonolysis followed by gas liquid chromatography (GLC) analysis of the fragments (14). Relative reaction rate constants were calculated by a digital computer according to a DRATE program written by Butterfield (15).

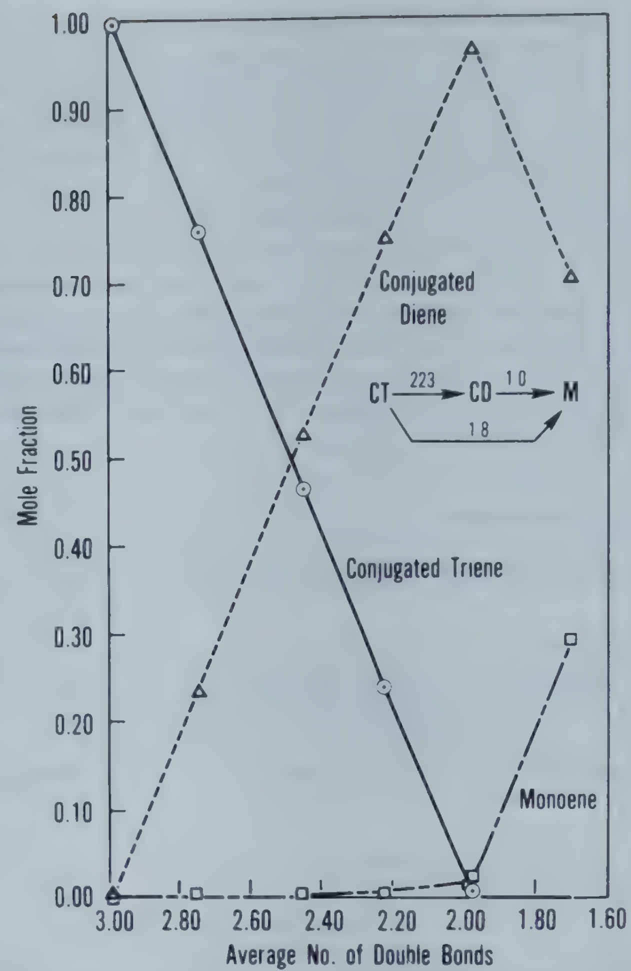


FIG. 1. Computer-drawn curves for the course of hydrogenation of β-eleostearate. CT = conjugated triene, CD = conjugated diene, M = monoene.

RESULTS AND DISCUSSION

Hydrogenation of Conjugated Trienes

If polyunsaturates conjugate prior to hydrogenation, information on the composition of products formed from conjugated esters will be useful to better understand the mechanism. β-Eleostearate reduced exclusively to conjugated dienes (Fig. 1), which did not further hydrogenate until all the conjugated triene disappeared. From these results conjugated triene was calculated to be over 200 times more reactive than conjugated diene. This high selectivity implies that the reduction of β-eleostearate over conjugated diene is nearly absolute. This is quite evident from Figure 1,, where hardly any monoene was formed when conjugated triene disappeared completely. Similar high selectivity for conjugated triene over conjugated diene was observed with copper chromite catalyst during atmospheric hydrogenation (16).

When 54% of β-eleostearate was reduced, the double bond distribution in the products shown in Figure 2 resulted. Conjugated dienes are formed either by 1,2- or 1,6-addition of hydrogen to the double bonds in β-eleostearate. Equal amounts of $\Delta^{9,11}$ and $\Delta^{11,13}$ conjugated diene isomers result by 1,2-addition, whereas 1,6-addition yields the $\Delta^{10,12}$ isomer. Nearly equal amounts of these 3 isomers are formed which indicates that 1,2-addition is preferred over 1,6-addition. Similar results were obtained during atmospheric hydrogenation (5). All 3 isomers could result by 1,4-addition of hydrogen followed by conjugation of the resulting $\Delta^{9,12}$ and $\Delta^{10,13}$ methylene-interrupted dienes. This possibility was discounted since methylene-interrupted dienes were not found. It also might be argued that the $\Delta^{10,12}$ isomer was solely the result of 1,2-addition of hydrogen to the $\Delta^{8,10,12}$ and $\Delta^{10,12,14}$ trienes which were in turn formed by isomerization. This is unlikely because the $\Delta^{10,12}$ isomer was found in large amounts even during early stages of hydrogenation (5% reduction) when $\Delta^{8,10,12}$ and $\Delta^{10,12,14}$ isomers were insignificant compared to $\Delta^{9,11,13}$ triene (17). Furthermore, $\Delta^{10,12}$ was formed with nickel and Lindlar catalysts that did not isomerize unreduced conjugated triene (18).

After 54% of β-eleostearate was reduced, 44% of the remaining conjugated trienes had double bonds in their original 9,11 and 13 positions. GLC analysis also indicated that geometric isomers formed. However, isomerization of β-eleostearate was much smaller at high pressure compared to atmospheric hydrogenation (5) where only 28% of the conjugated triene had double bonds at 9,11,13 positions after 50% reduction.

Hydrogenation of Conjugated Diene

During hydrogenation of alkali-conjugated linoleate (equimixture of *c*9,*t*11- and *t*10,*c*12-octadecadienoate), some *c,t*-conjugated diene isomerized to *t,t*-isomer (Table I). Positional isomers of conjugated diene also formed. For example, in the 5-min sample, only 68% of the double bonds were found in the original 9,11 and 10,12 positions, whereas 27% of the conjugated dienes had double bonds at 8,10 and 11,13 positions. The remainder had double bonds at 7,9 and 12,14 positions. Isomerization of conjugated dienes was, however, much less than that observed at atmospheric hydrogenation (5) where only 40% of the conjugated dienes had double bonds in the 9,11 and 10,12 positions after 50% reduction.

Hydrogenation of Linoleate

Hydrogenation of linoleate was accompanied by geometric isomerization (Table II). For example, after 58% reduction

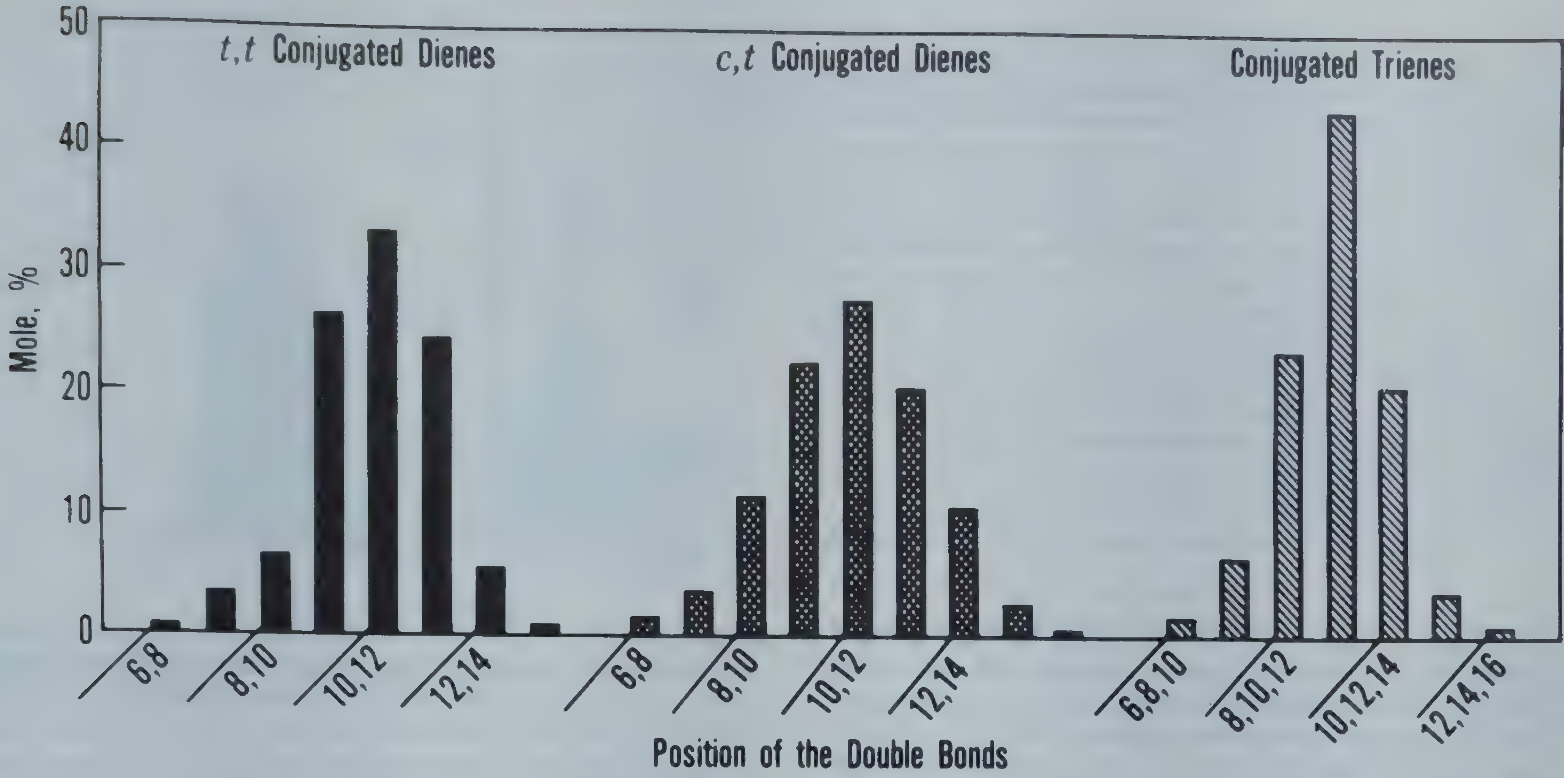


FIG. 2. Double bond distribution in various fractions formed during hydrogenation of β-eleostearate.

(59-min sample), 39% *trans* unsaturation was found in the remaining diene. In contrast, no *trans*-isomers of linoleate were formed at 30 psi (19). Some nonconjugatable diene was also formed during hydrogenation at high pressure. About 12% of the remaining diene in the 100-min sample (Table II) was not conjugatable. At low pressures (19), all remaining diene was conjugatable. Conjugated diene was not found in the products at high pressure, but up to 4% conjugated diene was found at 30 psi (19).

When methyl linoleate and alkali-conjugated linoleate were hydrogenated to the same extent (5- and 59-min samples of Tables I and II, respectively), the double bond distribution of the *trans*-monoenes (Fig. 3) was quite similar, which strongly suggests that the double bonds in linoleate conjugated prior to hydrogenation. The percentage of linoleate hydrogenated through a conjugated intermediate can be estimated by comparing the proportions of Δ^{10} and Δ^{11} *trans*-monoenes formed from linoleate to those from alkali-conjugated linoleate. According to this criterion, 95% of the double bonds in linoleate conjugated prior to hydrogenation. Recently Van Der Plank and Van Oosten (20) suggested plotting the sum of Δ^{10} and Δ^{11} isomers against the sum of Δ^{9} and Δ^{12} isomers from linoleate and alkali-conjugated linoleate and comparing the slopes. By this method, 85% of the double bonds in linoleate conjugated prior to hydrogenation.

TABLE I

Fatty Acid Composition of Products from Hydrogenation of Alkali-conjugated Linoleate with Copper-chromite Catalyst[a]

Me ester	Time (min) 0	2	5	8
Monoene	0.4	24.8	54.6	76.5
% *trans* in monoene	-	69.8	69.0	71.7
Diene	3.5[b]	3.4	3.8	3.2
c,t-Conjugated diene	93.2	48.9	15.4	7.1
t,t-Conjugated diene	2.8	22.9	26.1	13.2

[a]Catalyst (0.5%) at 170 C and 900-950 psig.
[b]Unreacted methyl linoleate.

TABLE II

Hydrogenation of Methyl Linoleate with Copper-chromite Catalyst[a]

Me ester	Time (min) 25	59	100
Monoene	39.2	57.8	69.2
% *trans* in monoene	56.1	58.8	59.2
Diene	60.8	42.2	30.8
% *trans* in diene	20.9	38.9	51.8
% Diene, conjugatable	95.5	94.1	88.1

[a]Catalyst (0.5%) at 170 C and 900-950 psig.

Hydrogenation of Methyl Linolenate

Hydrogenation of methyl linolenate with copper-chromite

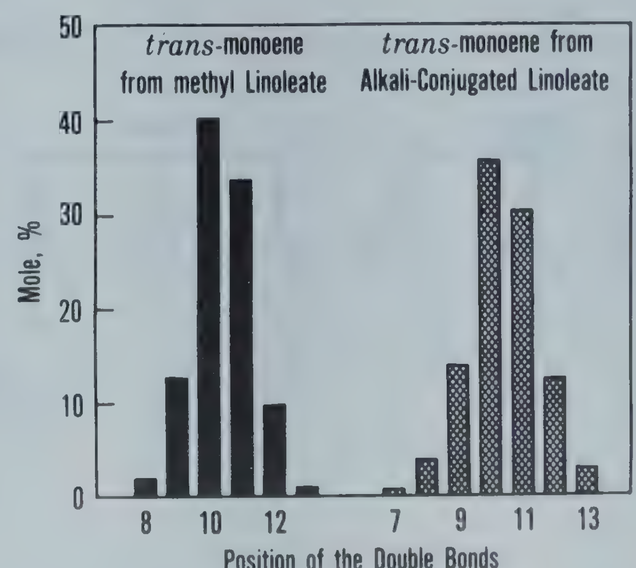


FIG. 3. Double bond distribution in *trans*-monoenes formed during hydrogenation of methyl linoleate and alkali-conjugated linoleate. Monoenes from 5- and 59-min samples, respectively, of Tables I and II.

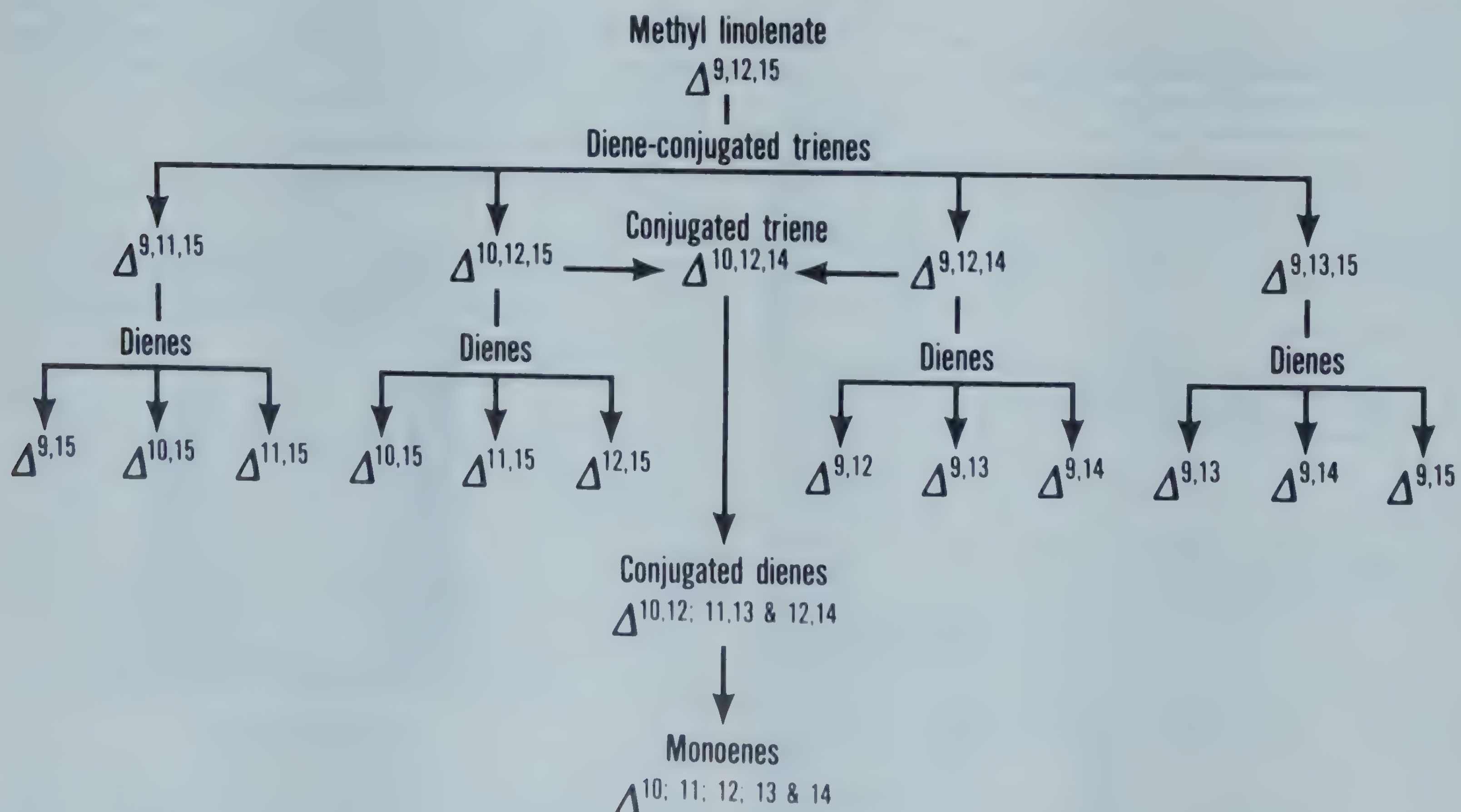
TABLE III

Fatty Acid Composition of Products Formed during Hydrogenation of Methyl Linolenate with Copper-chromite Catalyst[a]

Me ester	Time (min)					
	0	2	4	7	13	30
Palmitate	0.3	0.3	0.3	0.3	0.3	0.3
Stearate	0.1	0.2	0.2	0.2	0.1	0.3
Monoene	1.0	5.6	11.8	16.3	21.3	30.1
% *trans* in monoene	-	-	-	70.8	65.2	66.8
Diene	1.3	14.4	27.9	40.8	56.9	66.1
% *trans* in diene	-	-	-	49.3	50.3	62.9
% Diene, conjugatable	-	23.6	28.0	26.2	26.5	21.9
Triene	97.3	79.5	59.8	42.4	21.5	3.2
% *trans* in triene	-	-	-	23.8	48.2	-

[a]Catalyst (1%) at 170 C and 900-950 psig.

at high pressure (Table III) was accompanied by geometric isomerization. In 7- and 13-min samples, 24 and 48%, respectively, of the remaining triene had *trans*-unsaturation. Geometric isomerization was negligible during hydrogenation at atmospheric pressure (3). Conjugated dienes were absent at high pressure and conjugatable and nonconjugatable dienes were the major products. When all the triene disappeared (30 min), 30% monoene and 67% diene were formed. At low pressure, on the other hand, conjugated diene was the major product (3). After complete hydrogenation of triene at low pressure, 83% monoene and 16% diene were formed (4). Even though the products formed at high pressure were different from those at low pressure, conjugation prior to hydrogenation must still be the basic mechanism as shown in Scheme I. This scheme envisions the formation of conjugated intermediates that do not desorb from the catalyst surface until hydrogenated to nonconjugated products. Hydrogenation of diene-conjugated trienes results in both conjugatable and nonconjugatable dienes. Also, this mechanism predicts that a large proportion of the dienes should have their first double bond located at the 9,10-position. In fact, ozonolysis of the diene fraction from the 30-min sample (Table III) showed 62% of the dienes had their first double bond at the 9,10-position. The remainder of the dienes had their first double bond at 10,11- (12%), 11,12- (14%) and 12,13-positions (11%). Monoenes could form only through conjugated triene intermediates. It is true that conjugatable diene intermediates ($\Delta^{9,12}$ and $\Delta^{12,15}$) also form monoenes through conjugation. But since these dienes are much less reactive than triene (K_{Le}/K_{Lo} = 10), the monoenes contributed by conjugatable dienes compared to conjugated intermediates would not be significant. This mechanism predicts the formation of monoene isomers whose double bonds are at 10,11- through 14,15-positions. The double bond distribution in *trans*-monoenes (Fig. 4) from linolenate hydrogenation is consistent with the conjugation mechanism. The conjugation mechanism further predicts the formation of



SCHEME I

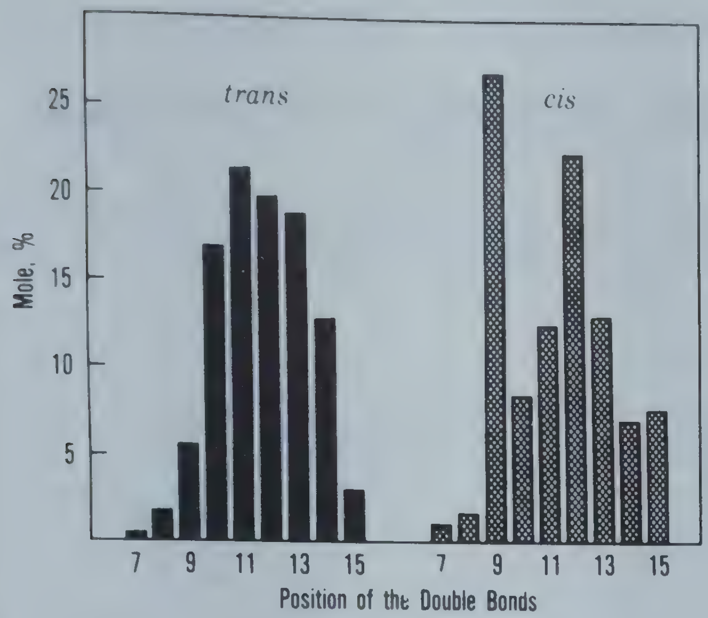


FIG. 4. Double bond distribution in *cis* and *trans* monoenes from methyl linolenate—7-min sample, Table III.

Δ^9, Δ^{12} and Δ^{15} *cis*-monoenes from conjugatable dienes. Surprisingly, Δ^{15}-*cis*-monoene was much smaller than the Δ^9 and Δ^{12} isomers (Fig. 4). This can be explained if we assume that $\Delta^{9,12}$ diene isomer is more reactive than the $\Delta^{12,15}$ isomer or that 1,2-addition of hydrogen to conjugated intermediates is preferential at the 15,16-position. Of course, no evidence exists for these assumptions.

Hydrogenation of methyl linolenate was simulated on a digital computer according to the scheme shown in Figure 5. The computer-drawn curves represent the best fit for the experimental points when the summed, squared error was at a minimum. The relative reaction rates shown in the inset were obtained. The scheme assumes the formation of monoene directly from linolenate without the conjugated triene and conjugated diene intermediates desorbing from the catalyst surface. Similarly, diene-conjugated triene is formed on the catalyst surface and will hydrogenate to either nonconjugatable (polymethylene interrupted) or conjugatable (single methylene interrupted) diene. The proposed scheme in Figure 5 is consistent with the conjugation mechanisms of Scheme I. The scheme in Figure 5 is, for the most part, similar to that proposed for low-pressure hydrogenation (4) except that at high pressure conjugated diene intermediates do not desorb from the catalyst surface because they are assumed to be much more reactive than single methylene-interrupted polyunsaturated fatty acids. The validity of this assumption was confirmed from the relative reaction rates of polyunsaturated fatty acids and their conjugated counterparts (Figs. 6-8).

Hydrogenation of Conjugated and Unconjugated Ester Mixtures

Computer simulation of the hydrogenation of conjugated diene and linoleate (Fig. 6) indicated that conjugated diene is 31 times more reactive than linoleate. At atmospheric pressure (4), where the concentration of hydrogen is lower at the catalyst surface, the relative rates were 9:1. Conjugated triene is over 100 times more reactive than methyl linolenate (Fig. 7) and similar high selectivity for conjugated triene was observed for low-pressure hydrogenation (4). Even conjugated dienes are 12 times more reactive than the triene, methyl linolenate (Fig. 8). At atmospheric pressure (4), conjugated diene was less than half as reactive as methyl linolenate.

The results of our study indicate that, with few exceptions, the mechanism of hydrogenation at high pressure (900-950 psi) is similar to that at low pressure (0-30 psi). Polyunsaturated fatty esters conjugate prior to hydrogenation both at low and high pressures. The same conjugation mechanism, therefore, explains the high selectivity at

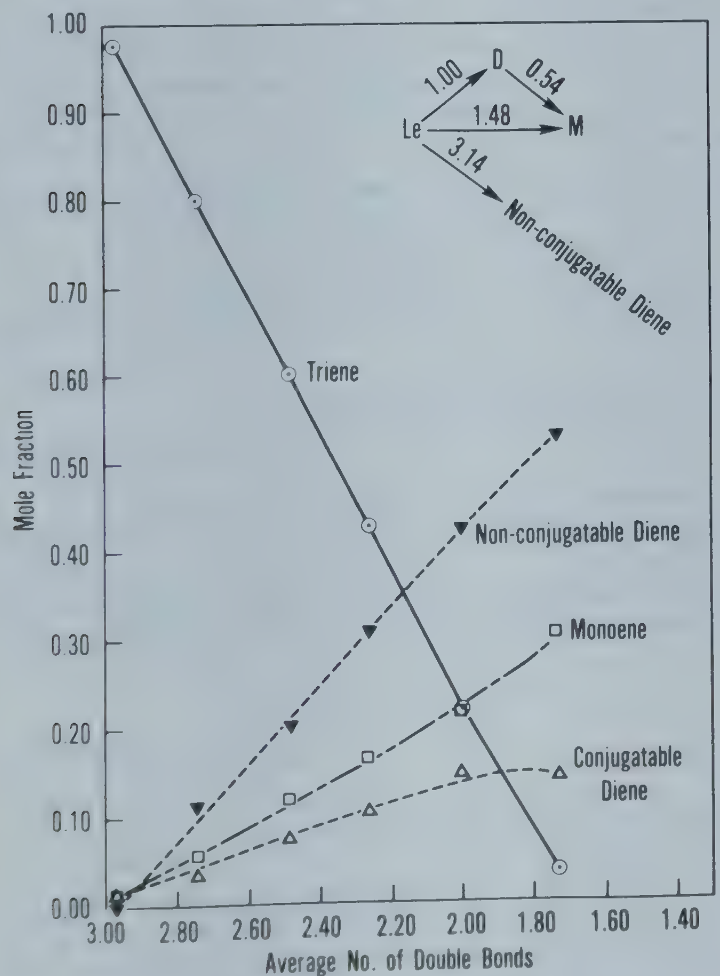


FIG. 5. Computer-drawn curves for the course of hydrogenation of methyl linolenate.

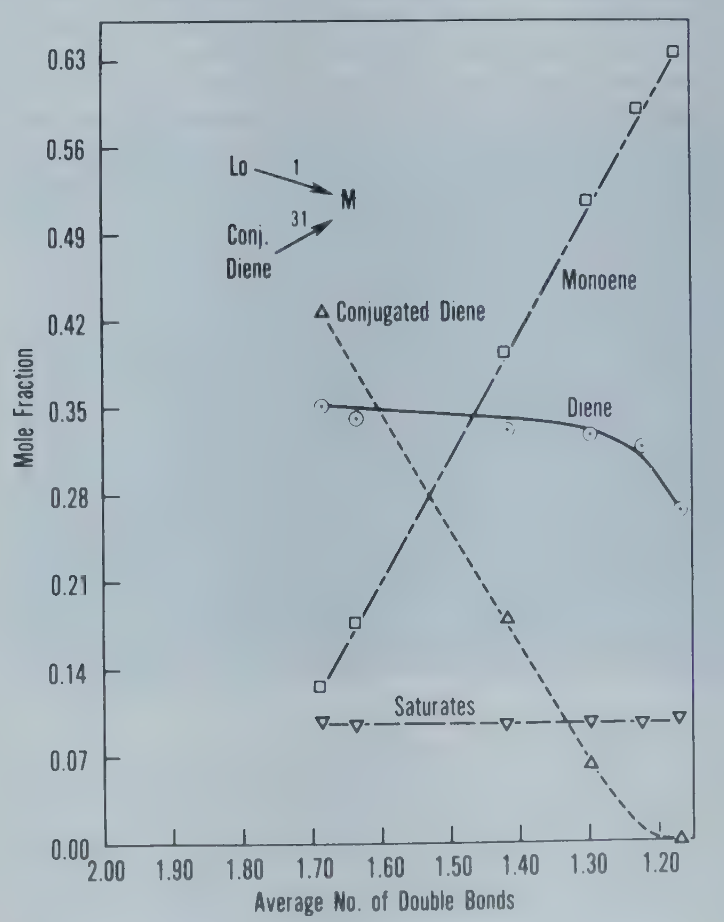


FIG. 6. Hydrogenation of methyl ester mixture of safflower and alkali-conjugated safflower oil.

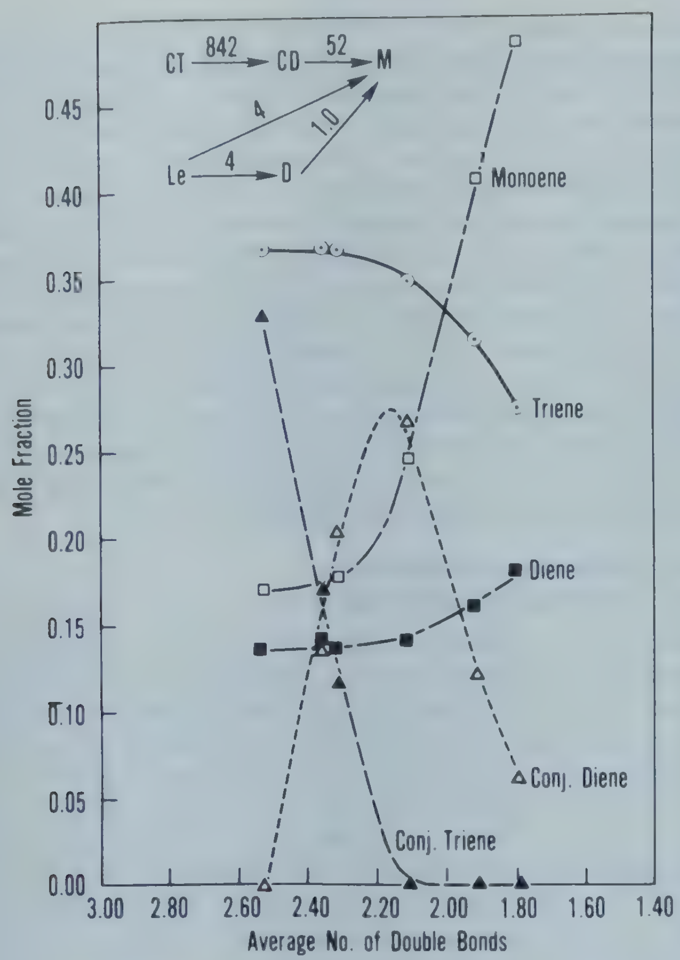


FIG. 7. Hydrogenation of methyl ester mixture of tung and linseed oil.

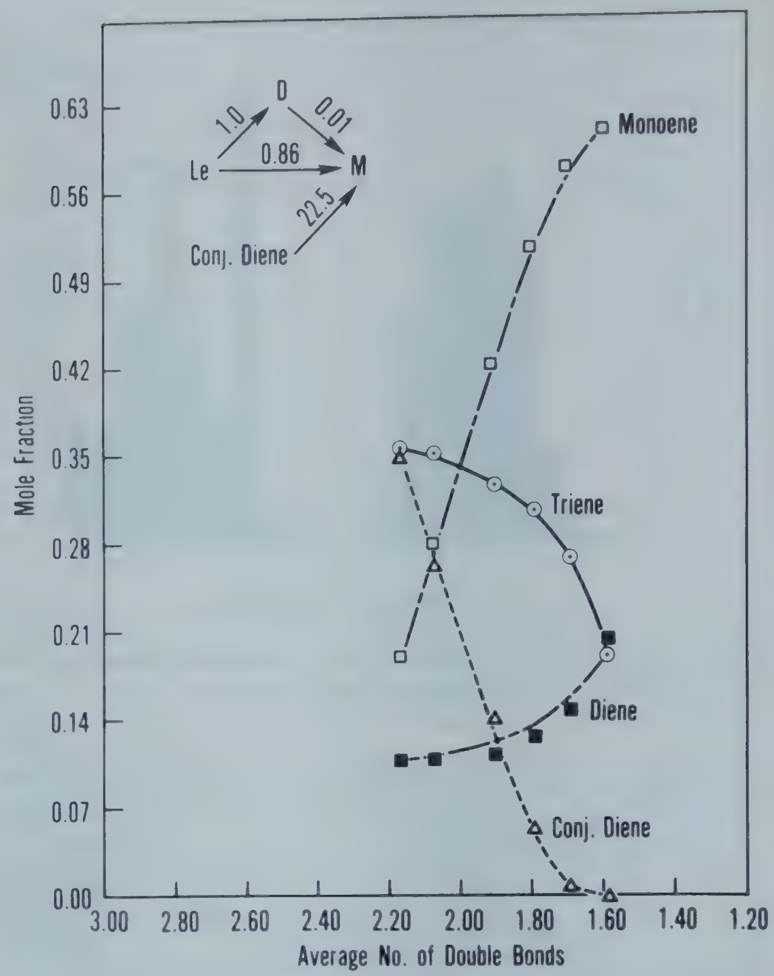


FIG. 8. Hydrogenation of methyl ester mixture of linseed and alkali-conjugated safflower oil.

high pressure (1). The mode of hydrogen addition to conjugated esters is similar at both pressure ranges although isomerization of conjugated esters was less at high pressure. Conjugated esters are preferentially hydrogenated over unconjugated esters at low and high pressures but more so at the higher pressure compared to the low pressure. In light of this observation, it is not surprising that conjugated dienes are absent from soybean oil hydrogenated at high pressures (1,21). One dissimilarity observed at the higher pressure is the isomerization of polyunsaturated esters to *trans* isomers. The increase in percentage of *trans* isomers at high pressures (21) primarily resulted from this isomerization reaction which was absent at the low pressure.

ACKNOWLEDGMENTS

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REFERENCES

1. Mounts, T.L., S. Koritala, J.P. Friedrich and H.J. Dutton, JAOCS 55:402 (1978).
2. Mattil, K.F., in "Bailey's Industrial Oil and Fat Products," 3rd Edition, edited by D. Swern, Interscience Publishers, New York, 1964, p. 823.
3. Koritala, S., and C.R. Scholfield, JAOCS 47:262 (1970).
4. Koritala, S., R.O. Butterfield and H.J. Dutton, Ibid. 47:266 (1970).
5. Koritala, S., Ibid. 47:269 (1970).
6. Butterfield, R.O., H.J. Dutton and C.R. Scholfield, Anal. Chem. 38:86 (1966).
7. Scholfield, C.R., R.O. Butterfield and H.J. Dutton, Lipids 1:163 (1966).
8. Hoffmann, J.S., R.T. O'Connor, D.C. Heinzelman and W.G. Bickford, Ibid. 34:388 (1957).
9. Radin, N.S., A.K. Hajra and Y. Akahori, J. Lipid Res. 1:250 (1960).
10. "Official and Tentative Methods of the American Oil Chemists' Society," Vol. I, 3rd Edition, AOCS, Champaign, IL, 1964 (revised to 1972), Method Cd 7-58.
11. Scholfield, C.R., Anal. Chem. 47:1417 (1975).
12. Hirsch, J., Colloq. Int. CNRS 99:11 (1961).
13. Emken, E.A., C.R. Scholfield and H.J. Dutton, JAOCS 41:388 (1964).
14. Johnston, A.E., H.J. Dutton, C.R. Scholfield and R.O. Butterfield, Ibid. 55:486 (1978).
15. Butterfield, R.O., Ibid. 46:429 (1969).
16. Koritala, S., R.O. Butterfield and H.J. Dutton, Ibid. 50:317 (1973).
17. Koritala, S., and E. Selke, Ibid. 48:222 (1971).
18. Koritala, S., C.R. Scholfield, E. Selke and H.J. Dutton, Ibid. 50:11 (1973).
19. Koritala, S., E. Selke and H.J. Dutton, Ibid. 50:310 (1973).
20. Van Der Plank, P., and H.J. Van Oosten, Ibid. 56:54 (1979).
21. Koritala, S., J.P. Friedrich and T.L. Mounts, Ibid. 57:1 (1980).

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A Conversion Factor to Determine Phospholipid Content in Soybean and Sunflower Crude Oils

G.W. CHAPMAN, Jr., Field Crops Laboratory, Richard B. Russell Agricultural Research Center, PO Box 5677, Athens, GA 30613

ABSTRACT

The major phospholipids (PL) from soybeans and sunflowers were separated by 2-dimensional thin layer chromatography (TLC) and the fatty acid composition of each PL was determined by gas liquid chromatography (GLC). PL from soybeans and sunflowers contained high percentages of linoleic and palmitic acids. Only phosphatidylinositol (PI) from both oilseeds were similar in fatty acid composition and the principal acid was palmitic acid. Sunflower phospholipids, except for PI, contained twice as much oleic acid as did those from soybeans. Sunflower PI contained very low but measurable quantities of heptadecanoic acid. The molecular weights (MW) of individual PL were based on their fatty acid composition. The MW found for soybeans and sunflower PL were quite similar even though their fatty acid compositions were different. The average MW of PL in crude soybean and sunflower oils was determined based on the MW of individual PL and their composition in the PL fraction. From that MW, a factor for converting phosphorous content in oil to its PL content was calculated. For both oils, the factor was 25.

INTRODUCTION

In several studies of developing and mature oilseeds, the polar lipid fraction or individual phospholipids (PL) were isolated and their fatty acid compositions were determined (1-8). The fatty acid data, however, were not used to determine the molecular weights (MW) of the individual PL. Such information, along with a knowledge of the PL composition of the oil, may enable a more realistic determination of PL contents of extracted crude oils on the basis of their elemental phosphorous content.

Factors are used to convert elemental phosphorous to PL content. The conversion factor for "acetone insolubles" in crude soybean oil is 30-31.7 (9,10) because it contains substantial amounts of nonphosphorous lipids, i.e., glycolipids and neutral lipids. However, the validity of this factor when used with degummed and refined oils has been questioned recently (11). On the other hand, the factor for other crude oils is given as 25.5 (see L.V. Cocks and C. Vanrede, A Laboratory Handbook for Fat and Oil Analysis, Academic Press, New York, 1966, pp. 137-147) and was calculated from a lecithin with an empirical formula $C_{44}H_{86}PO_9N$ (12).

The purpose of this study was to (a) determine more precise individual PL MW based on their fatty acid compositions, (b) use those results and the PL compositions of the oils to calculate their respective average PL MW and (c) use those weights to calculate conversion factors for soybean and sunflower crude oils.

MATERIALS AND METHODS

Crude oils were extracted from mature soybeans (mixed var.) and sunflower seed (var. Master Farmer) with chloroform/methanol (2:1) after they were ground in liquid nitrogen as previously described (13). The crude oil was centrifuged at 23,400 × g and the clear supernatant oil removed. The remaining pellet was rinsed several times with chloroform and dissolved in ca. 2 ml chloroform. Both the clear supernatant oil and dissolved pellet were used separately as PL sources for this study, the oil to determine its PL composition and the pellet to determine PL fatty acid composition.

Individual PL were separated by 2-dimensional thin layer chromatography (2D-TLC) on precoated Silica Gel 60 plates (14) and identified with specific spray reagents (15). Two samples (dissolved pellets from above) spotted separately (2.0 mg, 3 × 5 μl) on the lower left- and right-hand corners were developed on a single plate. Both samples were eluted with basic solvent (chloroform/methanol/7 N ammonium hydroxide, 65:30:4) for 10 cm; then, after drying with a hand-held dryer, the plate was rotated 90° and one of the samples was eluted with acidic solvent (chloroform/methanol/acetic acid/water, 170:25:25:4) for 9.0 cm. After drying, the plate was rotated 180° and the other sample eluted with the acidic solvent for 9.0 cm. Small amounts of neutral lipid and free fatty acids from the 2 samples overlapped; however, this did not affect the separation of PL (Fig. 1). Five to 6 such chromatograms yielded enough individual PL for fatty acid analysis. PL composition of crude soybean and sunflower oils were also obtained by a 3-solvent system, 2D-TLC, as previously described (13).

After visualization with iodine, each PL spot was carefully scraped into a 15 × 150 mm culture tube. Individual PL were saponified with 2.0 ml 0.5N $NaOH/CH_3OH$ at 83 C for 6 min without removal of the silica gel. Fatty acids were esterified with 3.0 ml BCl_3/CH_3OH (10%) at 83 C for 5.5 min. Fatty acid methyl esters were extracted from the reaction mixture with hexane and the vol reduced to

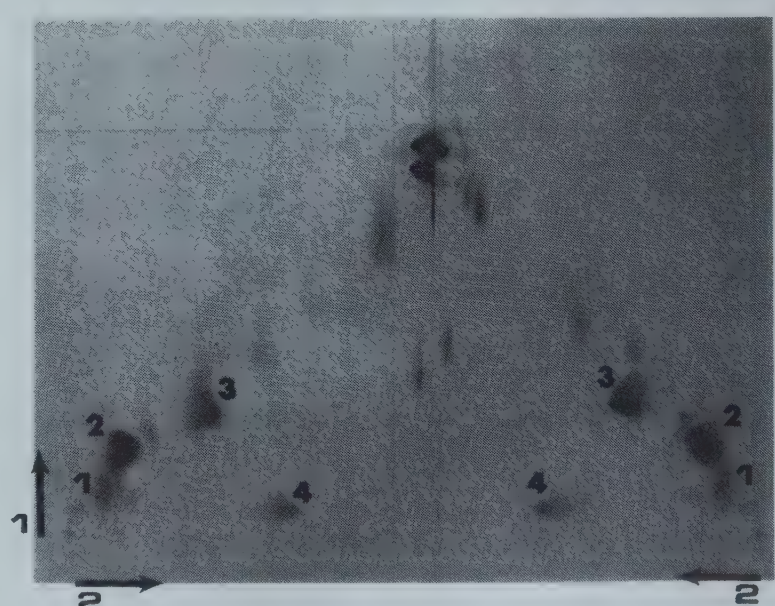

FIG. 1. 2D-TLC of 2 crude soybean lipid samples on one TLC plate. Origins are lower left- and right-hand corners. Basic solvent (#1), chloroform/methanol/7N NH_4OH, 65:30:4, from bottom to top. Acidic solvent (#2), chloroform/methanol/acetic acid/water, 170:25:25:4, from right to left and after drying, from left to right. Spots are identified as 1: phosphatidylinositol; 2: phosphatidylcholine; 3: phosphatidylethanolamine; 4: phosphatidic acid. Sunflower phospholipids migrated in the same way and plates were identical to the one shown.

TABLE I

Phospholipid Compositions of Soybean and Sunflower Crude Oils

Phospholipid	% Individual phospholipids in total phospholipids[a]				
	Soybeans			Sunflowers	
	Mixed var. (this study)	Harwood (1975)[b]	Privett (1973)[c]	Master Farmer var. (this study)	Borodulina (1974)
Phosphatidic acid	4.8	1.5	5.0	2.2	2.2
Phosphatidylinositol	20.3	17.5	14.0	27.9	24.0
Phosphatidylethanolamine	23.3	21.4	26.0	21.2	18.2
Phosphatidylcholine	39.0	43.6	45.0	48.7	55.4
UNK	12.5	4.3	6.3	-	-

[a]Based on P analyses of the individual and total phospholipids.

[b]Composition also included, cardiolipin-3.7%, phytoglycolipid-6.1%, *N*-acylphosphatidylethanolamine-1.1%.

[c]Composition also included, phosphatidyl glycerol-diphosphatidyl glycerol-3.3%.

TABLE II

Fatty Acid Composition of Major Phospholipids of Soybean and Sunflower[a]

Phospholipid	Fatty acid					
	16:0	17:0	18:0	18:1	18:2	18:3
Phosphatidylcholine						
Soybean	20.5 ± 0.5	-	5.5 ± 0.1	10.5 ± 0.3	58.8 ± 0.0	4.6 ± 0.3
Sunflower	18.2 ± 1.1	-	4.7 ± 0.1	23.5 ± 0.2	53.5 ± 1.3	-
Phosphatidylethanolamine						
Soybean	31.6 ± 1.6	-	3.2 ± 0.3	8.7 ± 0.9	53.2 ± 0.1	3.2 ± 0.5
Sunflower	29.6 ± 0.3	-	5.7 ± 0.2	17.4 ± 0.2	47.2 ± 0.3	-
Phosphatidylinositol						
Soybean	47.7 ± 1.5	-	8.2 ± 0.3	4.9 ± 0.5	36.2 ± 0.6	2.8 ± 0.6
Sunflower	45.4 ± 0.9	0.4 ± 0.0	10.5 ± 0.0	5.8 ± 0.1	37.8 ± 1.0	
Phosphatidic acid						
Soybean	34.0 ± 2.9	-	8.1 ± 0.5	11.9 ± 0.7	44.7 ± 1.4	1.3
Sunflower	34.2 ± 2.5	-	10.6 ± 0.5	22.4 ± 0.9	32.8 ± 3.3	-

[a]Area % ± SEM.

ca. 0.2 ml with a stream of dry nitrogen. Methyl esters were analyzed by gas liquid chromatography (GLC) using conditions previously described (16).

RESULTS AND DISCUSSION

Soybeans contained 7 PL as determined by 2D-TLC and molybdate spray reagent (Table I). Three PL were unidentified and accounted for 12.5% of the total. The data on soybean PL agreed well with Harwood results (17) and with those of Privett et al. (4). Only the 4 major PL identified in soybeans were used for fatty acid analysis. Only 4 PL were detected in sunflowers; their identities and relative distribution compared favorably with the findings of Borodulina et al. (18). Small amounts of phosphatidyl serine (PS) have, however, been reported in crude sunflower lecithin (3). PL from crude sunflower oil contained higher percentages of phosphatidylinositol (PI) and phosphatidylcholine (PC) than crude oil from soybeans, whereas the soybean crude oil contained higher percentages of phosphatidic acid, phosphatidylethanolamine (PE) and unknown PL.

The fatty acid compositions of the individual PL (soybean and sunflower) that remained in the supernatant oils and those PL that centrifuged down with the pellets were the same.

Linoleic acid was the major fatty acid in most of the PL analyzed (32-58%, Table II). A rather high percentage of palmitic acid was found in all soybean and sunflower PL (18-47%). Because palmitic and linoleic were the 2 major fatty acids in these oilseed PL, 1 mol of the most abundant PL molecular species would likely contain 1 mol each of palmitic and linoleic acids. Individual sunflower PL separated by LH-20 Sephadex chromatography were also found to contain high percentages of linoleic and palmitic acids (3). Generally, the percentage of palmitic acid is rather low in crude oils extracted from mature soybeans and sunflower seeds—11% and 5%, respectively (19,20). This is most likely because although PL contain high percentages of palmitic acid, they are generally found in crude oils at low levels (0.2-3.0%) and thus contribute very little to the total crude oil fatty acid profiles.

The fatty acid compositions of PI were quite similar between the 2 oilseeds whereas the fatty acid composition of the other PL differed (Table II). PC, PE and phosphatidic acid from sunflowers contained ca. twice as much oleic acid as did those from soybeans.

The molecular weights of individual PL from soybeans and sunflowers were computed based on their fatty acid compositions (Table III). PC from soybeans will serve to illustrate how these values were computed; data for the computation are presented in Table IV.

Average MW of one fatty acid/mol PC = Σ (molecular weight contribution all fatty acids). Av. MW of one fatty acid/mol PC = 274.5. MW of phosphocholine glycerol minus 2 hydroxyls = 223.2; then, molecular weight of PC = 2 × 274.5 + 223.2 = 772.2.

The av. MW of all PL in soybean and sunflower crude oils could then be determined based on the molecular weights of the individual PL and their relative composition of total PL (Table I): mol wt of all PL = Σ (decimal fract. of individual PL × MW of individual PL). In calculating the

TABLE III

Molecular Weights of Individual Phospholipids Based on Fatty Acid Composition[a]

Phospholipid	Soybean	Sunflower
Phosphatidic acid	681.6	681.2
Phosphatidylinositol	837.0	837.2
Phosphatidylethanolamine	724.4	725.8
Phosphatidylcholine	772.0	774.6

[a]Average of 2 determinations.

average MW of soybean PL, the unknown PL (12.5%) were assumed to be phosphatidylglycerol, since it has been identified as a component of soybean PL at mature stages of development (4,8). Furthermore, it was assumed that its fatty acid composition was similar to that of the other soybean PL fatty acids and its MW was thus computed 772.3. Its contribution to the av. PL MW was 96.5 MW units. The av. MW of soybean PL was computed to be 769.7, and that of sunflower PL, 779.5.

The av. MW of PL could be used to compute a factor for converting the percentage phosphorus (% P) in crude soybean and sunflower oils to the percentage PL. The source of all elemental phosphorous in crude oils is dissolved PL and since there is 1 mol P/mol PL, the relationship between % PL and % P in oils can be expressed by the following:

$$\% \text{ PL} = \% \text{ P} \times \frac{1}{\text{wt fract. P}} \qquad [\text{I}]$$

Calculating the wt fraction P in soybean oil from its average PL MW as just found:

$$\text{Wt fract. P} = \frac{\text{mol wt P}}{\text{mol wt PL}} = \frac{30.97}{769.7} = .0402$$

$$\text{And, } \frac{1}{\text{Wt fract. P}} = 24.8$$

For Sunflowers:

$$\text{Wt fract. P} = \frac{\text{mol wt P}}{\text{mol wt PL}} = \frac{30.97}{779.5} = .0397$$

$$\text{And, } \frac{1}{\text{Wt fract. P}} = 25.2$$

These values are fairly close and average 25.0; therefore, one equation, % PL = % P × 25, could be used to calculate the percentage of PL in both soybean and sunflower crude oils.

The % PL in crude, uncentrifuged, soybean oil was computed from the MW of its PL and compared to the value obtained using the equation with actual raw data.

Raw data:

Absorbance (A) of total-lipid phosphorous from sample at 820 nm = 2.351
Weight of total lipid sample applied to TLC plate = 13.5 mg
Based on standard curve with the phosphorus procedure (21), μmol P = A/2.75 and μg P = 11.26 × A
Av. MW of soybean PL = 769.7

$$\% \text{ PL} = \frac{\text{mg PL}}{\text{mg total lipid}} \times 100$$

$$= \frac{A/2.75 \times 769.7\ \mu\text{g}/\mu\text{mol}}{13.5\text{ mg}} \times 100$$

$$= 4.87$$

Using % PL = % P × 25:

$$\% \text{ P} = \frac{\text{mg P}}{\text{mg total lipid}} \times 100$$

$$= \frac{11.26 \times A}{13.5\text{ mg}} \times 100$$

$$= 0.196$$

Then: % PL = % P × 25 = 0.196 × 25 = 4.90.

For comparison, if we multiply by 30, then % PL = 5.88, a 20% higher value.

The 2 values (4.87 and 4.90) are in good agreement and indicate total % PL in crude oils can be determined by % P × 25, as well as from av. PL MW.

Since PL fatty acid composition and the composition of PL in crude oils were quite different between soybeans and sunflowers (Tables I and II), it is surprising that the av. PL MW were so similar (Table III) and yielded almost identical conversion factors. It is not known whether 25 or some other number would be obtained as the factor for other oilseeds, if the approach just described is used; however, the results of this study indicate that % P × 25 would give more realistic values of PL content in crude soybean and sunflower oils than the factor 30.

REFERENCES

1. Daga, H.G., Indian Chem. Jour. 11:21 (1976).
2. Kurucz, E., P. Biacs and A. Erdelyi, Olaj, Szappan, Kozmet. 24:100 (1975).
3. Biacs, P., J. Hollo, A. Erdelyi and E. Kurucz-Lusztig, The Sunflower Newsletter (International Sunflower Association) 3:27 (1979).
4. Privett, O.S., K.A. Dougherty, W.L. Erdahl and A. Stolyhwo, JAOCS 50:516 (1973).
5. Harwood, J.L., Phytochemistry 15:1459 (1976).
6. Slack, C.R., and P.G. Roughan, Biochem. J. 170:437 (1978).
7. Roughan, P.G., C.R. Slack and R.Holland, Lipids 13:497 (1978).
8. Wilson, R.F., and R.W. Rinne, Plant Physiol. 54:744 (1974).
9. List, G.R., A.J. Heakin, C.D. Evans, L.T. Black and T.L. Mounts, JAOCS 55:521 (1979).

TABLE IV

Data for Computing the Molecular Weight of Soybean Phosphatidylcholine (PC)

Fatty acid	FA composition of PC (as decimal)	FA MW (dissociated)	MW contribution of each FA[a]
16:0	0.205	255.4	52.4
18:0	0.055	283.5	15.6
18:1	0.105	281.4	29.5
18:2	0.588	279.4	164.3
18:3	0.046	277.4	12.8

[a]Weight contribution of each fatty acid = decimal fraction × MW fatty acid.

10. "Official and Tentative Methods of the American Oil Chemists' Society," AOCS, Champaign, IL (revised to 1973), Method Ca 12-55.
11. Hartman, L., JAOCS 56:908 (1979).
12. Jamieson, G.S., and R.S. Mckinney, Oil Soap (Chicago) 12:70 (1935).
13. Chapman, G.W., Jr. and J.A. Robertson, JAOCS 54:195 (1977).
14. Hitchcock, C. and B.W. Nichols in "Experimental Botany," Vol. IV, edited by J.F. Sutcliff and P. Mahlberg, Academic Press, London and New York, 1971, p. 287.
15. Skipski, V.P., and M. Barclay in "Methods in Enzymology," Vol. XIV, edited by J.M. Lowenstein, Academic Press, London and New York, 1969, pp. 542-548.
16. Chapman, G.W., Jr., JAOCS 56:77 (1979).
17. Harwood, J.L., Phytochemistry 14:1985 (1975).
18. Borodulina, A.A., D.S. Popov and I.N. Harchenko, Proc. VI International Sunflower Conference, July 22-24, 1974, Bucharest, Rumania, pp. 239-245.
19. Chapman, G.W., Jr., J.A. Robertson, D. Burdick and M.B. Parker, JAOCS 53:54 (1976).
20. Robertson, J.A., G.W. Chapman, Jr. and R.L. Wilson, Jr., Ibid. 55:266 (1978).
21. Rouser, G., A.N. Siakotos and S. Fleischer, Lipids 1:85 (1966).

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Utilization of Membrane-produced Oilseed Isolates in Soft-serve Frozen Desserts

J.T. LAWHON, N.H. GOLIGHTLY and **E.W. LUSAS,** Food Protein Research and Development Center, Texas A&M University, College Station, TX 77843

ABSTRACT

Consumption of frozen desserts in the United States has increased steadily in recent years. However, rising costs of milk solids-not-fat (MSNF) used in dessert formulas may cause manufacturers to consider less-expensive nondairy protein sources as an alternative with the resulting products labeled "nondairy". Use of soy protein isolates and concentrates as food ingredients is rapidly gaining acceptance in the United States. Glandless cottonseed and peanut protein isolates are expected to become available in the next few years. A membrane isolation process which employs ultrafiltration membranes to produce protein isolates directly from oilseed flour extracts has now been developed. Performance of these isolates in frozen desserts was assessed. Taste panel scores of dessert samples for color, odor, textures, flavor and overall acceptability were statistically analyzed. Results showed MIP soy isolate could replace MSNF (a) at the 80% level without flavor or texture loss, (b) at the 60% level without loss in overall acceptability and (c) at the 40% level without quality loss in color and odor. MIP peanut isolate replaced MSNF (a) at the 80% level without textural change, (b) at the 60% level without loss in overall acceptability or desirable flavor and odor and (c) at the 40% level without color loss. MIP cottonseed SP isolate was used to replace MSNF (a) at the 60% level without flavor loss, (b) at the 40% level with no textural changes and (c) at the 20% level without loss in overall acceptability. Based on these results, MIP oilseed isolates (especially soy and peanut) are a possible alternate source of protein for use in soft-serve frozen desserts to the replacement levels stipulated.

INTRODUCTION

Consumption of frozen desserts in the United States has increased steadily in recent years. This trend is expected to continue. However, the spiraling costs of milk solids-not-fat (MSNF) used in dessert formulas may cause manufacturers to consider less-expensive nondairy protein sources as an alternative. Products in which nondairy proteins are incorporated would have to be labeled "nondairy" since Federal standards permit only dairy proteins in ice cream, ice milk or mellorine.

Futch (1979), in a survey of frozen dessert manufacturers found that 94% of those responding considered price an important factor in maintaining frozen dessert sales (1). An analysis reported by Boehm (1976) showed household comsumption of frozen desserts to be responsive to changes in retail prices, especially in the short term (2).

The alternative to MSNF most frequently employed in frozen desserts to date has been whey solids. A number of investigations have been made to determine the effects of whey solids on ice cream and other dairy products (3-7).

In general, the investigators agreed that whey solids, especially solids from sweet wheys, could satisfactorily replace MSNF to the limits allowed by present Federal standards of identity and perhaps beyond. However, some reduction in quality was reported from loss of firmness and smoothness and from the appearance of a pinkish color when the colorant, annatto, was used in the cheese process generating the whey.

Grey (1979) cites a trend toward the use of nondairy products in the dairy industry (8). Garland et al. (1979) reported research in which defatted, glandless cottonseed flour, glandless cottonseed storage protein isolate, deglanded cottonseed flour, soy flour, soy protein concentrate and soy protein isolate were substituted for various levels of MSNF in a frozen dessert formula (9).

In the work to be described here, oilseed protein isolates produced from defatted soy, glandless cottonseed and peanut flour by industrial ultrafiltration (UF) and reverse osmosis (RO) membranes were evaluated as replacements for MSNF at levels of 20, 40, 60 and 80% in a soft-serve frozen dessert formula. A control in which none of the MSNF were replaced was also included in statistically designed experiments.

The oilseed isolates evaluated were produced by a membrane isolation process (MIP) developed by investigators at Texas A&M University's Food Protein Research and Development Center (FPRDC) (10-13). Using the MIP, protein is extracted from oilseed flours following conventional procedures. However, protein is ultrafiltered directly from the liquid extract instead of being removed by isoelectric precipitation as is conventionally done. MIP isolates possess functional and nutritional properties that differ from those of conventional isolates. Thus, the performance of MIP isolates in soft-serve frozen dessert was assessed using a sensory test panel and analytical and color measurements.

EXPERIMENTAL PROCEDURES

Preparation of Oilseed Isolates

Soy and peanut MIP isolates were prepared following the

procedure shown in Figure 1. Extractions of soy and peanut flours were made with filtered tap water (30:1 water-to-flour ratio by weight) adjusted to pH 9 and pH 8, respectively, with $Ca(OH)_2$. Extraction continued for 40 min at 55 C for soy flour and 60 C for peanut flour. The flour-water slurries were subsequently centrifuged to obtain extracts for ultrafiltering.

Cottonseed storage protein (SP) isolate was prepared by first extracting the nonstorage protein (NSP) from glandless cottonseed flour with filtered tap water (18:1 water-to-flour ratio) at 28.5 C for 40 min (Fig. 2). NSP extract was separated from insoluble residue by centrifugation, pasteurized and precipitated at pH 4 using HCl. NSP curd was separated from the NSP whey by centrifugation and spray-dried.

The insoluble residue from the initial extraction was reextracted with water adjusted to pH 9.5 using NaOH. After centrifugation to remove insolubles, SP extract was combined with the NSP whey, pasteurized and prefiltered to 100 μ for ultrafiltering.

Each extract was ultrafiltered using the internally-coated tubular UF system of Abcor, Inc., Wilmington, MA. The UF system was equipped with 22 sq ft of Abcor's HFM-180 noncellulosic membrane. Feed solutions were processed at the manufacturers' recommended pressure and flow rate. Feed temperature was maintained at 65 C throughout the processing cycle to give increased flux and prevent microbial buildup. A dilution technique was applied to further purify the retentate after a 4:1 vol reduction in the original feed. Dilution consisted of adding to the concentrated feed a quantity of filtered water equal to either 3 or 4 times its vol and reconcentrating it. The UF membrane retentate was spray-dried.

Other Formula Ingredients

Foremost Edible Lactose #305 was obtained from Foremost Foods, San Francisco, CA. Nonfat dry milk solids (NFDMS), Go! Southern Special Stabilizer, ice cream color (an alkaline extract of annatto seeds) and vanilla extract were procured through Lilly Ice Cream Co., Bryan, TX. Jersey cream (containing 39% fat) was purchased from a local dairy and granulated sugar from a local supermarket.

Preparation of Blends

Each oilseed isolate was blended with lactose to give a blend with the same protein content as the NFDMS used, i.e., 35.5%. To clarify, it should be stated that NFDMS as used herein refers to the commercial nonfat dry milk powder purchased. The MSNF used refers to the total milk solids-not-fat component of the formula mix. MSNF includes NFDMS plus solids from the Jersey cream.

Preparation of Mixes

The basic formula for the frozen dessert is shown in Table I in the column headed "Control". Ingredients in formulations containing MIP isolates at different replacement levels are also given in the table. To incorporate an oilseed isolate in the formula, oilseed protein-lactose blends were substituted on a protein-for-protein basis for the desired percentage of MSNF in the formula.

Dry ingredients in each mix except the sugar were blended and then divided into 3 approximately equal parts. Each portion was then blended with 250 ml tap water at 65 C using the fastest speed of a 7-speed blender. These dispersions were then carefully transferred to a 4000-ml stainless steel beaker. All containers used were rinsed with tap water at 65 C and the rinse water added to the contents of the beaker. The remaining formula water required, the cream, coloring and flavoring were then added. Next, the sugar was dissolved in the mixture.

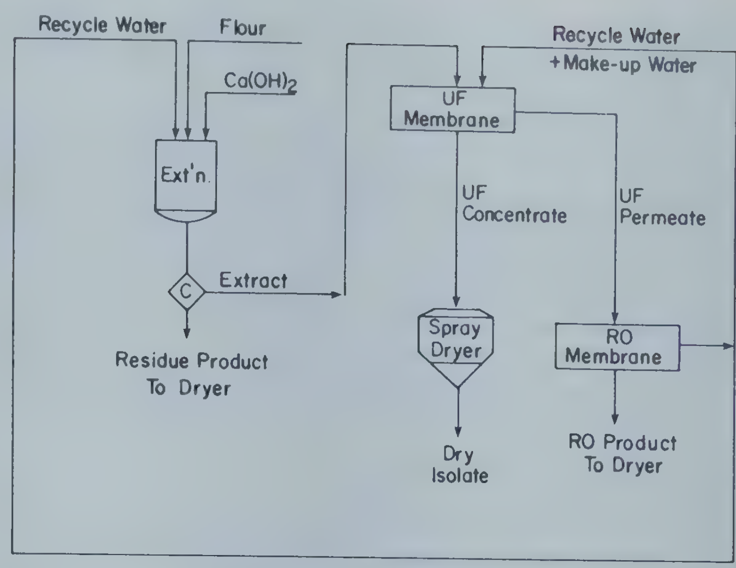

FIG. 1. Simplified flow diagram for soybean and peanut protein isolation with UF and RO membranes.

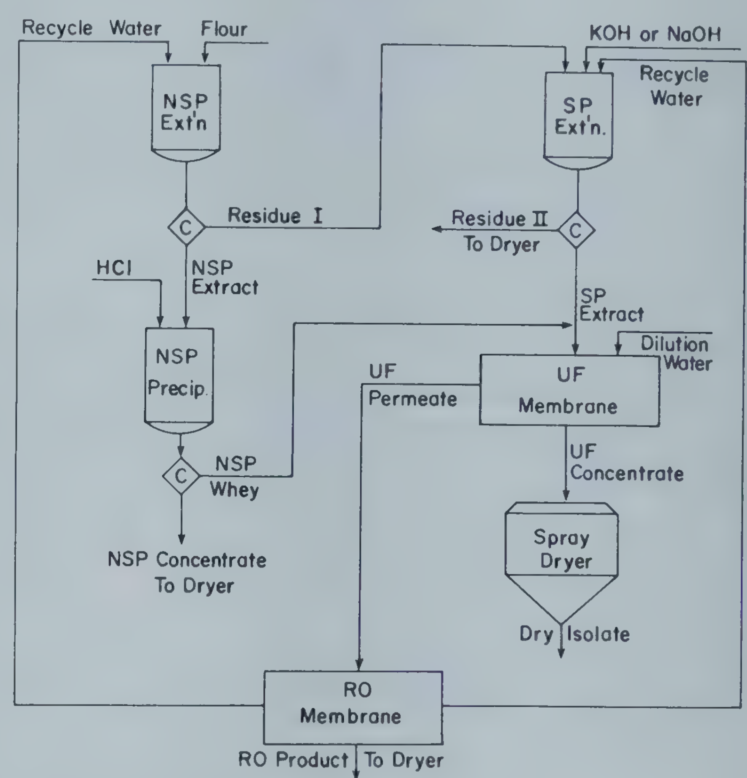

FIG. 2. Simplified flow diagram for cottonseed protein isolation with UF and RO membranes.

After preparation as described, beakers with mixes were placed in a water bath at 100 C until the mixes reached 78 C. Mixes were held at 78 C for 25 sec for pasteurization. They were then cooled to 65 C in a cold water bath while being stirred by hand. The cooled mixes were homogenized at 2500 psi using a Gaulin Type 15M homogenizer. They were recooled to around 4 C with an APV heat exchanger, Type Jr. H.E. Next, they were stored in a refrigerator at 4-6 C for aging for 5-7 days.

Measurements to Evaluate Mixes

Upon completion of storage for aging, 2.4 ℓ of each mix were placed in the freezing chamber of a Swedette Model A500 soft-serve freezer and frozen until the freezer automatically stopped. The temperature of the frozen dessert at this point was -7 C. The freezer automatically maintained the dessert at approximately this temperature.

Mix viscosity was then measured at 6 C using a Brook-

field Model LVT-E Viscometer equipped with T-Shaped Spindle A at 60 rpm. The pH of the mixes were subsequently read at 10 C.

Sensory evaluations were made of each frozen oilseed-protein-containing mix and the control. One mix was removed from storage, frozen and served to an 8-judge, semitrained taste panel at each session. After each mix had been scored, the series was replicated using newly prepared batches of mix. This gave 16 judgements on each dessert. Each sample was scored for texture, color (i.e., color acceptability not color difference), flavor, odor and overall acceptability, using this 5-point scale: 5-very good; 4-good; 3-fair; 2-poor; 1-very poor.

Statistical Design and Analyses

Thirteen treatment combinations (including the control) were randomized and mixes corresponding to them were prepared and stored under refrigeration. Treatment combinations were formed from 3 products (soy, glandless cottonseed SP and peanut isolate) at 4 levels (10, 40, 60 and 80%) plus a control treatment.

An analysis of variance was performed on each set of scores (odor, color, texture, flavor and overall acceptability) from the sensory evaluation. The GLM procedure of the SAS 76D package was followed in the computation (14). Sources of variation shown to be significant by analysis of variance were further tested using Duncan's New Multiple Range Test (15).

Analytical Procedures

Moisture, oil, crude fiber and ash were determined on oilseed isolates according to standard AOCS methods (16). Nitrogen was determined by the Kjeldahl method. Protein was calculated as nitrogen multiplied by 6.25. Nonprotein nitrogen (NPN) was determined as that nitrogen soluble in 10% TCA solution.

Total sugars in terms of glucose were measured colorimetrically by the Dubois et al. phenol-sulfuric acid method (17). Total phosphorus was determined by Sumner's method (18).

Isolate color measurements were made using the Model D25D2 Hunterlab Digital Color and Color Difference Meter. Measurements were first made with isolates in a dry form and then as a wet paste prepared by adding water (5:1, water/isolate, w/w). L-Scale readings (reflecting lightness of color) were taken.

Color measurements on desserts were made after the dessert had been frozen for 60 min. Colors (L, a, b scales) were read on the Hunterlab Meter Model D25D2) used with the isolates.

RESULTS AND DISCUSSION

Analytical and color data on MIP isolates tested in experimental frozen desserts are given in Table II. The peanut isolate is shown to have a protein content in excess of 100% because the 6.25 multiplier, though commonly used, is inappropriate for peanuts (19). The soy isolate was lighter in color in a dry state than either peanut of cottonseed isolate. However, when wetted, peanut isolate maintained a lighter color. Cottonseed SP isolate was the darkest of the 3 whether dry or wet.

The ingredients of mixes containing oilseed isolates at 4 replacement levels are shown in Table I along with the ingredients of the control. Different amounts of each isolate were required because of variation in their protein contents.

Mix viscosity, color and pH measurements are shown in Table III. Mixes containing isolates were less viscous than

TABLE I

Ingredients in Soft-serve Frozen Desserts Containing MIP and a Milk Protein Control

	MIP oilseed isolates and protein replacement levels												
	Soy (%)				Cottonseed (%)				Peanut (%)				
Ingredients[a]	20	40	60	80	20	40	60	80	20	40	60	80	Control
Water (g)	3636	3631	3627	3622	3638	3634	3631	3628	3637	3633	3630	3626	3640
NFDMS (g)	617.5	458.0	298.5	139.0	617.5	458.0	298.5	139.0	617.5	458.0	298.5	139.0	777.0
Lactose (g)	97.1	194.2	291.3	388.3	98.2	196.3	294.5	392.6	103.6	207.3	310.9	414.6	—
Oilseed isolate (g)	67.2	134.3	201.4	268.6	64.7	129.4	194.1	258.7	60.1	120.1	180.2	240.3	—

[a]Ingredients and amounts common to each mix: cream-838.2 g; sugar-814.5 g; stabilizer-29.7 g; vanilla-11.6 ml; food coloring -2.6 ml.

TABLE II

Data on MIP Oilseed Isolates Used in Soft-serve Frozen Desserts

Isolates	Ash	Nitrogen Total	Nitrogen NPN	Protein (N X 6.25)	Total P	Total sugars	Crude fiber	Oil	Color (L-scale) Dry	Color (L-scale) Wet
	% Dry wt basis									
Soy	7.1	14.59	0.60	91.19	0.95	5.6	0.2	0.9	82.5	59.5
Glandless cottonseed[a]	5.0	14.94	0.63	93.36	0.88	5.6	0.5	0.4	66.7	49.8
Peanut	3.2	16.23	0.29	101.39	0.23	2.5	0.1	0.4	79.2	67.3

[a]Storage protein extract plus nonstorage protein whey.

TABLE III

Properties of Soft-serve Frozen Desserts Containing MIP Oilseed Isolates and of a Milk Protein Control

Protein replacement level (%)	Protein source	Mix viscosity, CPS (6.0 C)	Color (frozen) L	Color (frozen) a	Color (frozen) b	pH
20	Soy	162	86.8	1.9	19.3	6.96
	Glandless cottonseed	164	83.4	1.9	19.0	6.72
	Peanut	155	85.7	2.2	19.3	6.73
40	Soy	199	83.9	1.2	17.6	7.09
	Glandless cottonseed	201	82.1	1.1	18.2	6.86
	Peanut	200	87.4	1.6	17.3	6.85
60	Soy	257	85.6	1.8	18.1	7.13
	Glandless cottonseed	253	78.4	2.6	21.0	6.98
	Peanut	323	87.2	1.2	16.6	6.89
80	Soy	272	84.5	2.2	17.5	7.18
	Glandless cottonseed	330	75.7	1.5	17.5	7.12
	Peanut	325	82.7	1.2	16.7	7.06
0 (Control)	Milk-solids-not-fat	260	87.4	2.1	21.0	6.65

TABLE IV

Mean Sensory Scores Assigned to Soft-serve Frozen Desserts Containing MIP Oilseed Isolates and to a Milk Protein Control[a]

Protein replacement level (%)	Protein source	Taste panel scores: Color means	Odor means	Texture means	Overall acceptability means	Flavor means
20	Soy	$4.02^{b,c}$	$3.90^{b,c}$	$3.92^{b,c}$	$3.96^{b,c}$	$3.81^{b,c,d}$
	Glandless cottonseed	3.63^{c}	3.63^{c}	$3.88^{b,c,d}$	$3.69^{b,c,b}$	$3.63^{b,c,d}$
	Peanut	$4.08^{b,c}$	$3.95^{b,c}$	$3.76^{b,c,d}$	$3.95^{b,c,d}$	$4.20^{b,c}$
40	Soy	$3.88^{b,c}$	$3.69^{b,c}$	$4.00^{b,c}$	$3.75^{b,c,d}$	$3.69^{b,c,d}$
	Glandless cottonseed	3.63^{c}	$3.75^{b,c}$	$3.63^{b,c,d}$	$3.31^{d,e}$	$3.31^{b,c,d}$
	Peanut	$3.81^{b,c}$	$3.75^{b,c}$	$4.06^{b,c}$	$3.94^{b,c,d}$	$3.56^{b,c,d}$
60	Soy	3.74^{c}	$3.56^{c,d}$	$3.80^{b,c,d}$	$3.86^{b,c,d}$	$3.42^{b,c,d}$
	Glandless cottonseed	2.94^{d}	$3.56^{c,d}$	$3.50^{c,d}$	$3.38^{c,d,e}$	$3.50^{b,c,d}$
	Peanut	3.71^{c}	$3.73^{b,c}$	$3.75^{b,c,d}$	$3.77^{b,c,d}$	$3.40^{b,c,d}$
80	Soy	3.68^{c}	3.63^{c}	$3.75^{b,c,d}$	$3.42^{c,d}$	$3.23^{b,c,d}$
	Glandless cottonseed	2.56^{d}	3.06^{d}	3.25^{d}	2.79^{e}	2.96^{d}
	Peanut	3.62^{c}	$3.55^{c,d}$	$3.55^{b,c,d}$	$3.35^{c,d,e}$	$3.08^{c,d}$
0 (Control)	Milk solids-not-fat	4.38^{b}	4.19^{b}	4.19^{b}	4.13^{b}	4.31^{b}

[a]Means of ca. 16 judge scores.

[b-e]Means with the same letter are not significantly different at the 5% level of significance.

the control at the 20, 40 and 60% replacement levels with the exception of the mix with peanut at the 60% level. Mixes containing peanut isolate were essentially equal in color to the control at the 40 and 60% replacement levels. Other mixes with isolates were not as light-colored as the control. The SP cottonseed product adversely affected color to a greater degree than did soy or peanut isolates. The presence of oilseed isolates in mixes tended to raise their pH slightly.

Mean sensory scores assigned to frozen desserts by panel members are presented in Table IV. Statistically analyzed mean scores are shown for color, odor, texture, flavor and overall acceptabiltiy.

The data show that up to 40% of the MSNF could be replaced with MIP soy or peanut isolates without loss of color lightness. Glandless cottonseed SP isolate measurably lowered the color even at the 20% replacement level. Odor scores indicate that soy-containing desserts did not significantly differ from the control through the 40% replacement level and that peanut-containing desserts equaled the control through the 60% replacement level. Odor scores for desserts with cottonseed were unexplainably higher at the 40% level than at the 20% level. No textural changes occurred in desserts having soy or peanut replacements through the 80% level. Cottonseed isolate affected texture adversely beyond the 40% replacement level.

Flavor scores show dessert flavor was unaffected by using soy through the 80% replacement level. Cottonseed and peanut flavor scores were not significantly different through the 60% level of replacement. The data also show that samples containing soy and peanut isolates at levels through 60% did not differ in overall acceptability from 0% replacement control samples. The cottonseed storage protein isolate did not affect overall acceptabiltiy at the 20% replacement level. Based on these results, MIP oilseed isolates are a possible alternate source of protein for use in frozen desserts when incorporated at replacement levels shown not to cause loss of acceptability.

ACKNOWLEDGMENTS

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REFERENCES

1. Futch, M., Dairy Ice Cream Field 162:48 (1979).
2. Boehm, W.T., Ibid. 159:58A (1976).
3. Arbuckle, W.S., Am. Dairy Rev. 34:15 (1972).
4. Singleton, A.S., Dairy Ice Cream Field 156:30 (1973).
5. Arnold, R.G., T.A. Evans and C.L. Kreshel, Ibid. 159:55 (1976).
6. Bhusri, A.S., and W.K. Jordan, Ibid. 160:77 (1977).
7. Hansen, A.P., Ibid. 162:53 (1979).
8. Grey, R.S., Ibid. 162:54 (1979).
9. Garland, R.J., J.R. Green, C.A. Payne, P.J. Wan and E.W. Lusas, Presented at the 39th Annual Meeting of the Institute of Food Technol., St. Louis, MO, June 1979. Submitted for publication in J. Food Sci.
10. Lawhon, J.T., D. Mulsow, C.M. Cater and K.F. Mattil, J. Food Sci. 42:389 (1977).
11. Lawhon, J.T., D.W. Hensley, D. Mulsow and K.F. Mattil, Ibid. 43:361 (1978).
12 Lawhon, J.T., D.W. Hensley, Masahiko Mizukoshi and D. Mulsow, Ibid. 44:213 (1979).
13. Lawhon, J.T., L.J. Manak and E.W. Lusas, Ibid. 44: (1980).
14. Barr, A.J., J.H. Goodnight, J.P. Sall and J.T. Helwig, "A User's Guide to SAS, 76" Sparks Press, Raleigh, NC, 1976.
15. Steel, R., and J. Torrie, "Principles and Procedures of Statistics," McGraw-Hill Book Company, Inc., 1960.
16. "Official and Tentative Methods," 3rd Edition, American Oil Chemists' Society, Chicago, IL, 1971.
17. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, Anal. Chem. 28:350 (1956).
18. Sumner, J.B., Science 100:413 (1944).
19. Watt, B.K., and A.L. Merrill, Agriculture Handbook No. 8, Agricultural Research Service, USDA, p. 161.

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The HPLC Separation and Quantitation of Lecithin in Chocolate

W.J. HURST and **R.A. MARTIN, JR.,** Technical Center, Hershey Foods Corporation, Hershey, PA 17033

ABSTRACT

A method is described for the analysis of phosphatidylcholine (chemical lecithin) in chocolate products. The chocolate is extracted with a modified Folch reagent and interfering compounds eliminated with a Sep-pak^{TM}. The extract is chromatographed using normal phase chromatography with a solvent system of $CH_3CN/CH_3OH/H_2O$ and detection at 210 nm. The method shows good recovery from soy lecithin and chocolate-type matrices. It also shows good precision with % Cv ranging from 1-8% depending on the matrix. Studies of the effects of lecithin on the rheological behavior of chocolate during processing can now be easily performed, as well as the monitoring of lecithin ingredient levels in chocolate products.

INTRODUCTION

Lecithin is used in chocolate as an emulsifier and surface active agent. It is extracted from soybeans by leaching with solvent. Technically, chocolate is a dispersion of fine solid particles in a fat matrix. In milk chocolate, milk is added to the other solid components. Lecithin's major effect is that of a viscosity reducer with other minor effects and the ability to employ higher processing temperatures without viscosity changes (1).

Lecithin analysis is an important need in the chocolate industry both as an ingredient check and as a means to monitor rheological phenomena throughout processing.

This soy lecithin (1) consists of 3 major phosphatides: 19.7% phosphatidylcholine (PC) (chemical lecithin), 19.7% phosphatidylethanolamine (PE) (cephalin) and 21.0% phosphatidylinositol (PI). In this study we chose to analyze lecithin for chemical lecithin rather than for all of the phospholipids.

Lecithin analysis traditionally has been performed by an extraction of acetone insolubles with phosphorus determination on the resulting fraction (2). This technique is time-consuming and results are difficult to reproduce.

Phospholipid analysis has also been made by liquid chromatography, using various supports such as silica gel, TEAE-cellulose and DEAE-cellulose with TLC on the resulting fraction (3-9). High performance liquid chromatography (HPLC) for the analysis of phospholipids has not

FIG. 1. Phosphatidylcholine standard (20 μg). Column: μPorasil (Waters Assoc.); mobile phase: 65:21:14 $CH_3CN/MeOH/H_2O$; flow rate: 2 ml/min; detector: Waters Assoc. Model 401 Refractive Index 8x.

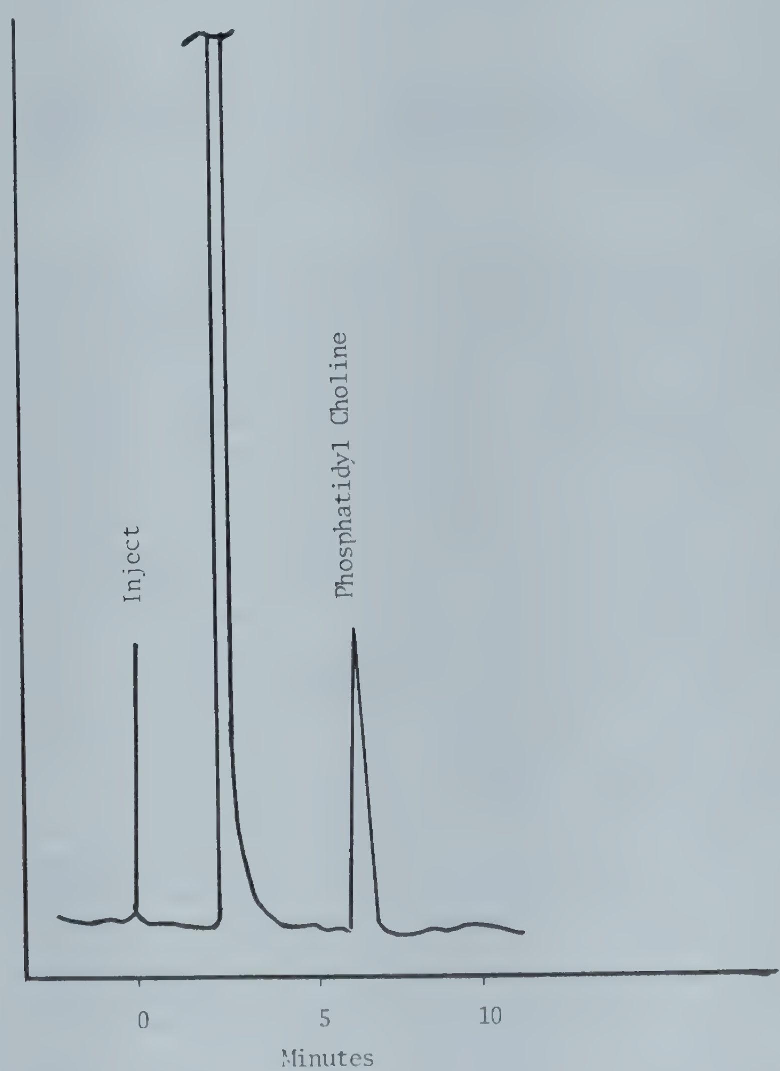

FIG. 2. Phosphatidylcholine standard (2 μg). Column: μPorasil (Waters Assoc.); mobile phase: 65:21:14 $CH_3CN/MeOH/H_2O$; flow rate: 2 ml/min; detector: Waters Model 450 @ 210 nm (.01 AUFS).

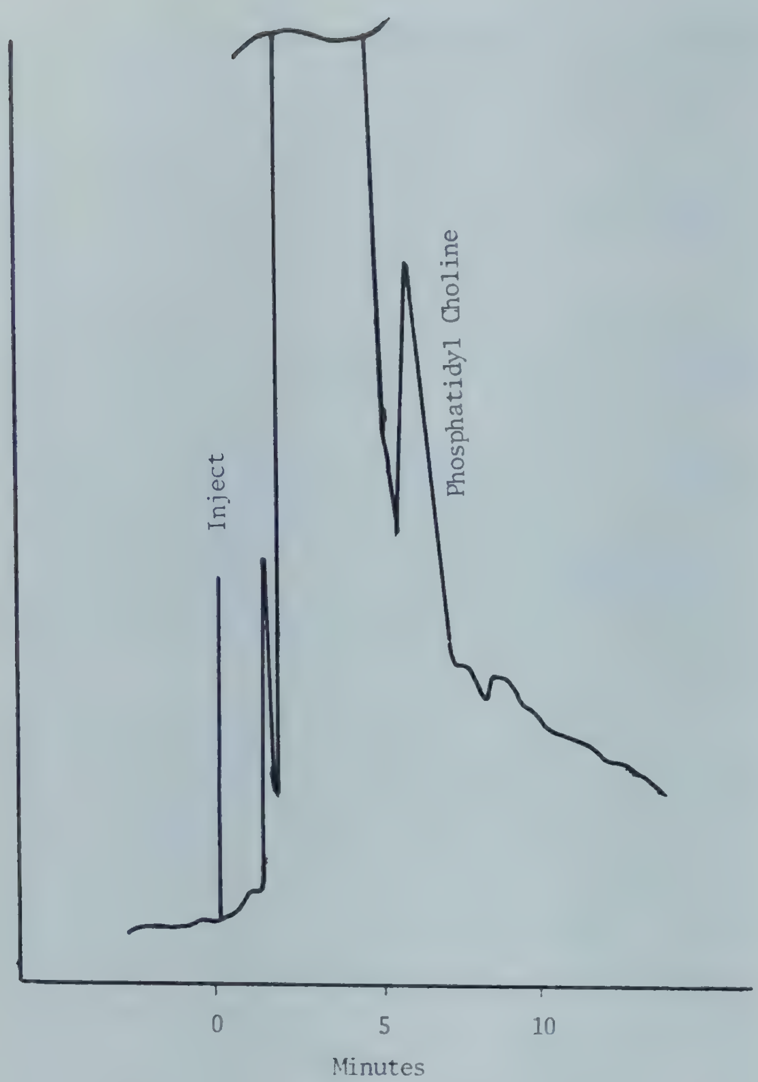

FIG. 3. Chocolate extract without Sep-pak™ clean up. Column: μPorasil (Waters Assoc.); mobile phase: 65:21:14 $CH_3CN/MeOH/H_2O$; flow rate: 2 ml/min; detector Waters Model 450 @ 210 nm (0.01 AUFS).

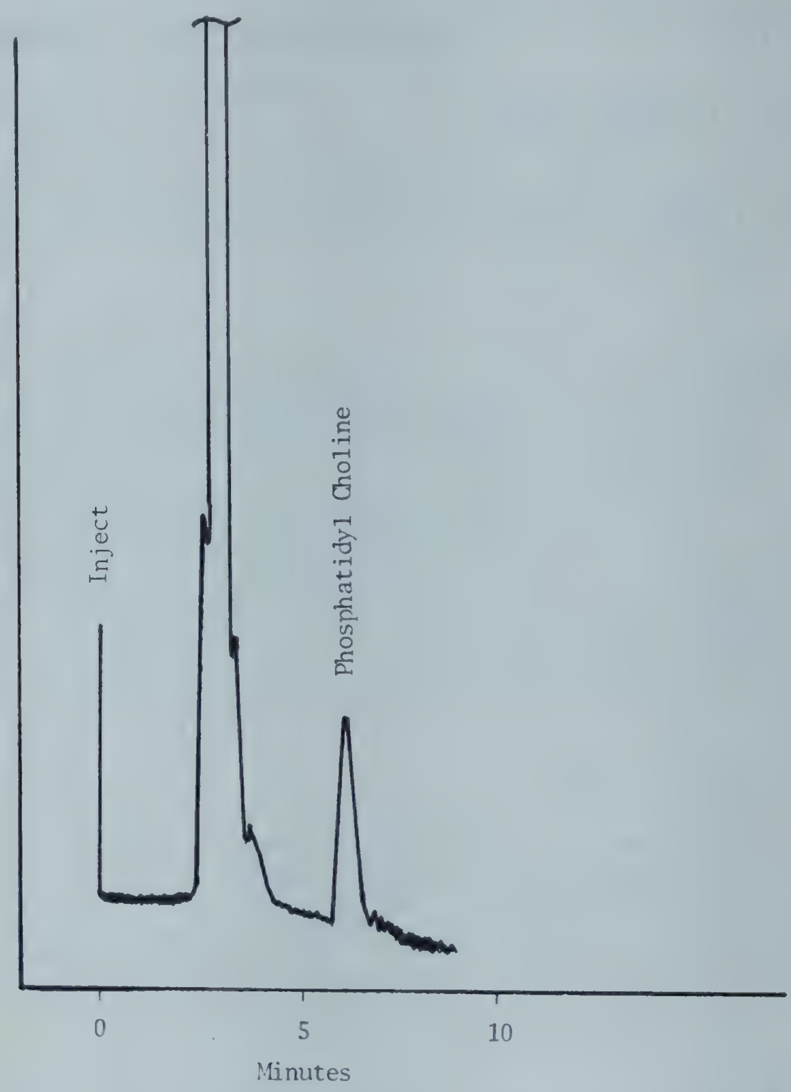

FIG. 4. Chocolate extract with Sep-pak™ clean up. Column: μPorasil (Waters Assoc.); mobile phase: 65:21:14 $CH_3CN/MeOH/H_2O$; flow rate: 2 ml/min; detector: Waters Assoc. Model 401 Refractive Index (4x).

been very successful since both refractive index (RI) and flame-ionization detection methods are insensitive. The phospholipids do not have strong absorption peaks but do have unsaturated centers and functional groups which exhibit absorption in the 203-214 nm region (10). It has been reported that this absorption primarily results from isolated double bonds with detector response proportional to the degree of unsaturation (11).

In this paper, a method is described for the analysis of chemical lecithin, PC, in chocolate using normal phase HPLC with detection at 210 nm. Samples are extracted and interferents eliminated using a commercially available Sep-pak™. The method is accurate, precise and is time-conservative; analysis time is less than 30 min.

EXPERIMENTAL PROCEDURES

HPLC

The HPLC apparatus consisted of an M45 or M6000A Solvent Delivery System (Waters Associates), a Porasil column (4.8 nm id x 30 cm) (Waters Associates), 2 detectors, a data unit and miscellaneous ancillary equipment. The 2 detectors used were an M401 Refractive Index Detector (Waters Associates) and a Model 450 Variable Wavelength Detector at 210 nm (Waters Associates). Data acquisition and evaluation were performed by the Shimadzu E-1A Data Unit. The HPLC mobile phase was $CH_3CN/CH_3OH/H_2O$ (65:21:14, v/v/v) at a flow rate of 2.0 ml/min. All solvents were LC grade.

Samples and Standards

Soy lecithin and chocolate samples were obtained at the Hershey Chocolate Company, Hershey, PA. Different lots of soy lecithin were obtained compliments of A.E. Staley Mfg. Co. All other samples were made at Hershey Research Laboratories, Hershey, PA. The standard used was L-α-lecithin from soybeans. (Sigma Chemical Co.)

Extraction of Samples

All samples were extracted using a modified Folch reagent (12) consisting of $CHCl_3:CH_3OH$ (2:1) plus BHT added at the 0.005% level to prevent oxidative degradation of unsaturated lipids (8). Soy lecithin samples were extracted and injected without further purification. Chocolate samples are extracted and the extract further purified using the silica gel Sep-pak™. Ten g of ground chocolate were extracted with 2 100-ml portions of modified Folch. The resulting solution was centrifuged and the resulting $CHCl_3/CH_3OH$ poured into a round-bottomed flask. This solution was evaporated under vacuum at 35-40 C until only the fat remained. The fat was dissolved in 25 ml of $CHCl_3$ and 10 ml of this solution passed through a silica gel Sep-pak™. The Sep-pak™ was washed with 10 ml of 7% petroleum ether in ethyl ether. The PC was eluted with 30 ml of CH_3OH and subjected to analysis.

Analysis

Samples and standards were injected onto the column in duplicate. Calculation of results was obtained by comparing

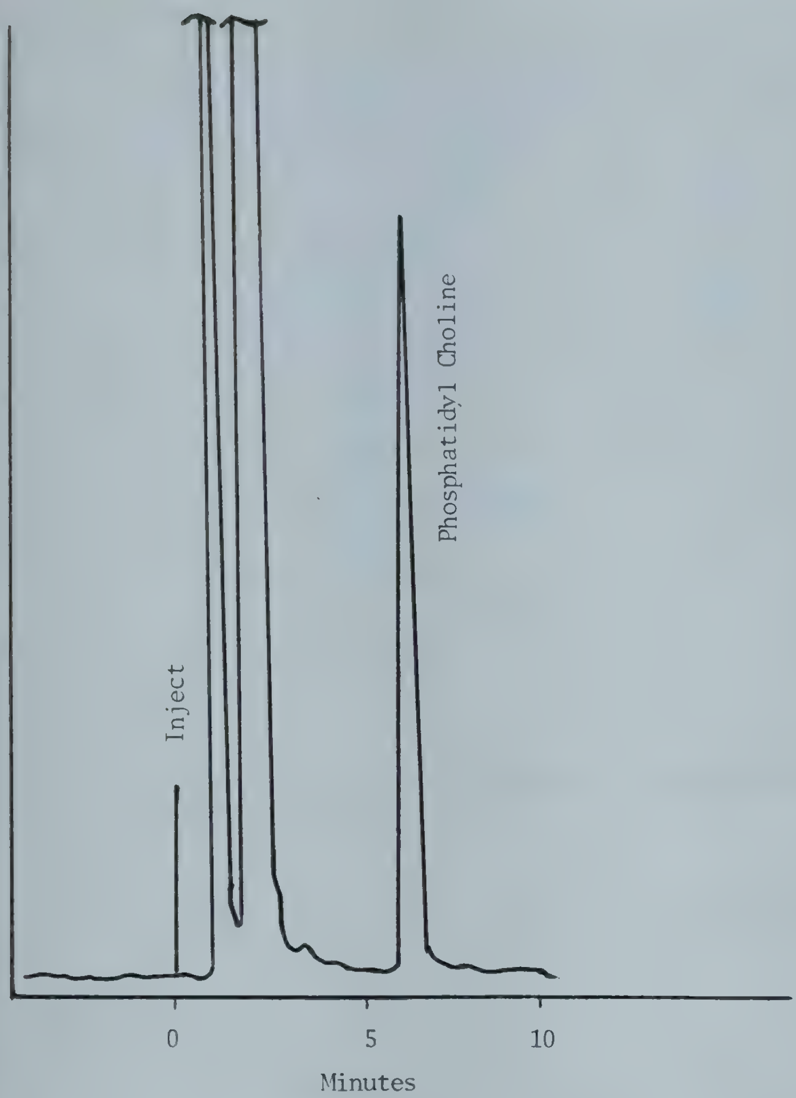

FIG. 5. Chocolate extract with Sep-pak™ clean up. Column: μPorasil (Waters Assoc.); mobile phase: 65:21:14 $CH_3CN/MeOH/H_2O$; flow rate: 2 ml/min; detector: Waters Model 340 @ 210 nm (0.01 AUFS).

peak areas obtained by injection of samples and standards. Figures 1 and 2 show sample chromatograms of lecithin standards using UV and RI detectors whereas Figure 3 shows a chromatogram of a chocolate extract without the Sep-pak™ clean up. Figures 4 and 5 show chocolate extract chromatograms with Sep-pak™ clean up with UV and RI detection.

Thin Layer Chromatography (TLC)

Ten μl of Folch extracts were spotted on silica gel TLC plates (Si-60; E. Merck). The plates were developed with $CH_3Cl_3/CH_3OH/HOAC/H_2O$ (25:15:4:2) (10). The phospholipids were visualized using either 10% ethanolic phosphomolybdic acid followed by exposure to heat of 110 C or exposure to I_2 vapors.

RESULTS

Six different manufacturers' lots of the same type of soy lecithin were analyzed for PC. The results are reported in Table I; the lot-to-lot coefficient of variation was less than 7%.

Table II shows the precision studies for analyses of different matrices with % Cv ranging from ca. 1 to 8%.

Duplicate recovery studies were conducted on 2 matrices, commercial lecithin and matrix consisting of 50% cocoa butter, 40% sugar and 10% cocoa. Table III shows good recovery from commercial lecithin and Table IV shows good recovery from the second matrix.

Cocoa liquor has indigenous PC; it was therefore necessary to elucidate the percentage of PC in various liquors used in chocolate products.

With the information on the choline content of soy lecithin used in the product and information on indigenous PC levels in chocolate liquor, it is therefore possible to calculate the the amount of lecithin in the finished product. Lower limits of detection are ca. 150 ng within the matrix and data are linear from 150 ng to over 4 μg.

TABLE I

Phosphatidylcholine in Lecithin Lots

Phosphatidylcholine (%)
27.31
26.43
28.64
23.91
24.70
27.88
$\bar{x}$ = 26.49
% Cv = 6.98

TABLE II

Precision Studies

Sample	n	% Cv
Phosphatidylcholine standard (soy)	10	4.34
Commercial soy lecithin	9	1.08
High liquor milk chocolate extract	10	1.99
Liquor extract	9	7.79
Low liquor milk chocolate extract	5	5.48

TABLE III

Recovery Study of Phosphatidylcholine Standard Added to Commercial Lecithin (n = 2)

Amount added (%)	Amount recovered (%)	Recovery (%)
+ 2	1.93	96.6
+ 5	4.83	96.5
+10	10.26	102.6
		Average 98.6

TABLE IV

Soy Lecithin Added to Cocoa Butter, Sugar and Cocoa Matrix

Amount added (g)	Amount recovered (g)	Recovery (%)
0.04571	0.04534	99.19
0.10260	0.09238	90.32
0.41350	0.50530	98.41
1.00240	0.92380	92.16
1.50690	1.36670	90.64
		Average 94.14

DISCUSSION

The data generated show the utility of this technique as an analytical tool. It allows the monitoring of ingredients and observations of various rheological phenomena to which lecithin is related. Additionally, without the use of the Sep-pak™ this would not be possible, since the chocolate extract is not sufficiently clean to permit analysis. It is

extremely important that the variable wavelength UV be used since the RI detector, while a universal detector, is only ca. 10% as sensitive as the UV. Finally, it would be possible to analyze for PE and other phospholipids, although they do elute extremely close to the solvent front using this mobile phase.

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REFERENCES

1. Minifie, B.W., Chocolate, Cocoa and Confectionery: Science and Technology, Avi Publishing Co., 1970.
2. Official Methods of Analysis, 12th Edition, edited by W. Horwitz, 1975, p.218.
3. Rouser, G., G. Kritchevsky, A. Yamamoto, G. Simon, C. Galli and A.J. Bowman, Methods Enzymol. 14:272 (1969).
4. Lipid Chromatographic Analysis, 2nd Edition, Vol. 3, edited by G. Rouser, G. Kritchevsky and A. Yamamoto, Marcel Dekker, New York, 1976, p.713.
5. Weenick, R.O., and A.P. Tullock, JAOCS 43:327 (1965).
6. Blood Lipids and Lipoproteins, edited by G.J. Nelson, Wiley-Interscience, New York, 1972, pp. 3-24.
7. Shipski, V.P., and M. Barclay, Methods Enzymol. 14:530 (1969).
8. Aitzetmuller, K., J. Chromatogr. 113:231 (1975).
9. Dieffenbacher, A., and U. Bracco, JAOCS 55:642 (1978).
10. McCluer, R.H., and F.B. Jungalwala, Adv. Exp. Med. Bio. 76: 533 (1976).
11. McCluer, R.H., and F.B. Jungalwala, Chromatogr. Sci.79:10 (1979).
12. Folch, J., M. Lees and G.H.S. Stanley, J. Biol. Chem. 226: 497 (1957).

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The Properties of *Cucurbita foetidissima* Seed Oil

J.A. VASCONCELLOS, J.W. BERRY and **C.W. WEBER,** Department of Nutrition and Food Science, University of Arizona[1], Tucson, AZ, and **W.P. BEMIS,** and **J.C. SCHEERENS,** Department of Plant Science, University of Arizona, Tucson, AZ 85721

ABSTRACT

Oils from the seeds of 15 different selections of the buffalo gourd, *Cucurbita foetidissima,* were characterized in terms of their physical and chemical properties, which indicate that this oil is similar to other common edible oils. Xanthophylls were the predominant carotenoid pigments present in the crude oil, ranging from 51-232 mg/kg oil. Linoleic acid, the predominant fatty acid, ranged from 39-77% with an average level of 61%. Although conjugated unsaturated acids are a significant component in some other xerophytic cucurbit oils, the levels of conjugated dienoic and trienoic fatty acids in this species are only 2.3 and 0.03%, respectively.

INTRODUCTION

The buffalo gourd, *Cucurbita foetidissima* HBK, a feral xerophytic gourd, has been the subject of a number of recent studies (1-5) because of its potential as a source of oil, protein and starch. The domestication of this plant as a crop adapted to arid land agriculture is currently under study (1). Previous investigation of the crude oil from the seed suggested that it could be processed to yield an edible oil (6). Its highly unsaturated nature should make it very attractive for food purposes.

The purpose of this work was to determine the physical and chemical properties of the crude oil from the seeds of this xerophytic plant.

EXPERIMENTAL PROCEDURES

Open-pollinated seed lots from 15 genetically different selections of *C. foetidissima* grown at the University of Arizona Experiment Station were studied. The seeds were obtained by submerging the fruits in water until fermentation disintegrated the placental tissue. They were then washed, air-dried and kept at room environmental conditions. Seed aliquots were taken as needed for different analyses, thus assuring that the determinations were performed on freshly extracted oil.

[1] Arizona Agricultural Experiment Station No. 3081.

Seed and Oil Analyses

Seed aliquots were ground in a laboratory Wiley mill to 10-mesh size through a nickel-plated delivery tube. Moisture content was obtained by drying in a vacuum oven for 16 hr at 60 C. Crude oil content was determined with 5 g samples by Soxhlet extraction with hexane; crude protein content of defatted meal was measured by the micro-Kjeldahl method using a conversion factor of 6.25.

Oil for physical and chemical analyses was extracted as already described. The hexane solution was filtered and the oil recovered in a rotary evaporator. Residual hexane was removed by heating the sample to 60 C while flushing the oil with a stream of N_2. Characterization of the oil samples was made using AOCS methods (7).

Total Carotenoids: Visible Spectrophotometry

Carotenoids were determined using matched silica cells of 1-cm path length read in a Perkin-Elmer 202 spectrophotometer. Oil samples were dissolved in cyclohexane (2.5% w/v) and the spectra recorded in the range 350-550 mμ. For

TABLE I

Composition of *Cucurbita foetidissima* Seed

Properties	Range	Mean ± SD
Moisture (%)	4.1 - 8.4	6.2 ± 0.2
Oil (%)	31.8 - 39.4	36.0 ± 2.0
Protein content[a], defatted meal (%)	49.0 - 66.0	54.6 ± 4.6

[a]Crude protein = % N × 6.25.

TABLE II

Properties of *Cucurbita foetidissima* Oil

Properties	Range	Mean ± SD
Refractive index		
at 25 C	1.4692 - 1.4747	1.472 ± 0.001
at 40 C	1.4652 - 1.4686	1.467 ± 0.001
at 60 C	1.4524 - 1.4603	1.456 ± 0.002
Free fatty acid (%)	0.3 - 0.8	0.5 ± 0.2
Acid value (%)	0.5 - 1.7	1.1 ± 0.4
Peroxide value (mg/kg oil)	8.5 - 44.9	16.9 ± 10.9
Saponification value	190.1 - 194.8	191.5 ± 1.6
Iodine value	123.0 - 138.0	129.9 ± 5.3
Acetyl value	-	7.3 (pooled)
Hydroxyl value	-	7.3 (pooled)
Specific gravity (25°/25 C)	-	0.9172 (pooled)
Carotenoids (mg/kg oil)	51.3 - 231.5	109.7 ± 55.0
Phosphatides (%)	0.3 - 1.2	0.8 ± 0.3
Minerals (ppm)		
P	95.0 - 390.	260.0 ± 100.0
Fe	0.38 - 1.11	0.68 ± 0.22
Cu	0.16 - 0.51	0.31 ± 0.12
Mn	0.09 - 0.37	0.24 ± 0.08

quantitative determination the absorbance was read at 417 mμ and the Zscheile et al. equation was used:

$$\text{mg carotene/kg oil} = \frac{(\text{absorbance at 417 m}\mu)\ (\text{sample volume, ml})}{0.204 \times (\text{sample weight, g})}$$

Conjugated Fatty Acids: Ultraviolet Spectrophotometry

To determine conjugated dienoic acid content, cyclohexane solutions (0.05% w/v) were examined in the region 200-260 mμ; similar solutions were used to detect conjugated trienoic and tetraenoic acids in the region 210-230 mμ.

The percentages of dienoic, trienoic and tetraenoic conjugated fatty acids were calculated according to AOAC Methods of Analysis (9).

Trans Fatty Acids: Infrared Spectrophotometry

Absorption spectra were obtained with a Perkin-Elmer 337 Grating Infrared spectrophotometer using NaCl cells with a film thickness of 0.5 mm. Samples were prepared as 2% solutions (w/v) in carbon disulfide. Concentration of the *trans* fatty acids was expressed as the percentage of trielaidin.

RESULTS AND DISCUSSION

The seeds upon extraction with hexane yielded ca. 36% oil, whereas the protein content of the remaining defatted meal was ca. 55%. The recovered oil had a bland taste and odor and the color varied from a dark reddish-brown to a light greenish-yellow in the seed lots examined.

Physical and chemical properties of the oil are summarized in Tables I and II. Certain properties, along with visible spectral characteristics, are compared with those of some common edible oils in Table III and Figure 1, respectively. These data show that *C. foetidissima* oil closely resembles other common oils which are processed for food use.

The level of carotenoids found in *C. foetidissima* oil was similar to that in cottonseed oil, ranging from 51.3-231.5 mg/kg, with a mean of 110 (Table II). Xanthophylls apparently are the predominant pigments present, as shown by absorption in the 400-420 mμ region (Fig. 1). In contrast, α- and β-carotenes predominate in red palm and soybean oils (10), and the spectrum of the β-carotenes is much less intense in the region indicated.

The mean and range for each fatty acid constituent measured in the 15 selections examined are shown in Table IV. Linoleic acid averaged 61% of the total fatty acids.

TABLE III

Characteristics of Various Crude Vegetable Oils[a]

	Soybean	Cottonseed	Corn	Safflower	*Cucurbita foetidissima*
Oil in seed (%)	21.0	22.9	4.5	30.0	36.0
Free fatty acid (%)	0.5	0.7	1.5	0.4	0.5
Acid value (%)	1.0	1.4	3.0	0.8	1.1
Iodine value	126.0	105.0	128.0	145.0	129.9
Sapon. value	193.0	195.0	190.6	191.0	191.5
Phosphatides (%)	2	1	2		0.8
Carotenoids (mg/kg oil)	40.0	167.0			109.7
Conjugated fatty acids (%)					
Dienoic	0.3	0.8			2.3
Trienoic	0.1	0.3			3.3 N 10^{-2}
Tetraenoic					0.0
Refractive index (25 C)	1.4730	1.4700	1.4720	1.4750	1.4720
Specific gravity (25°/25 C)	0.919	0.917	0.9175	0.921	0.9172

[a]Data for soybean, cottonseed, corn and safflower oils from Ref. 14.

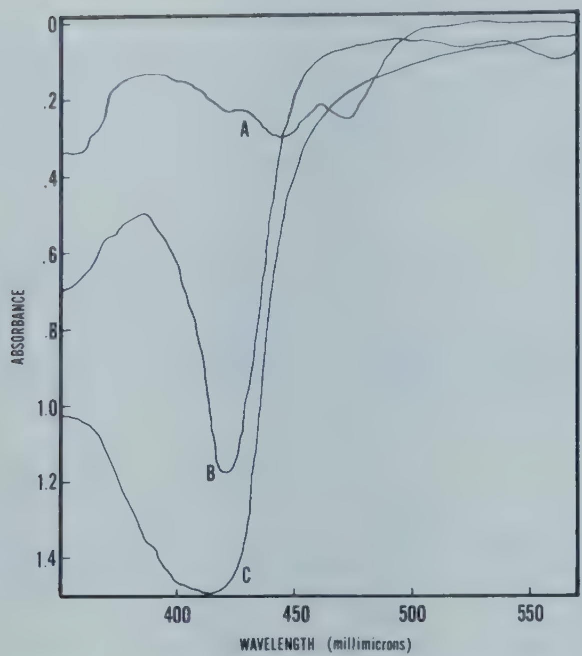

FIG. 1. Visible spectra of the crude oils from soybean, cottonseed and *C. foetidissima* in cyclohexane: A—soybean oil (2.9% w/v); B—*C. foetidissima* oil (2.507% w/v); C—cottonseed oil (2.528% w/v).

TABLE IV

Fatty Acid Content of *Cucurbita foetidissima* Oil

Fatty acids	Range	Mean ± SD
Major fatty acids (%)		
Palmitic	6.6 - 24.4	11.8 ± 4.8
Stearic	1.2 - 10.2	3.5 ± 2.2
Oleic	10.0 - 31.6	2.19 ± 6.2
Linoleic	39.3 - 77.2	60.6 ± 11.0
Conjugated fatty acids (%)		
Dienoics	1.9 - 2.8	2.3 ± 0.3
Trienoics	0.0 - 0.10	0.033 ± 0.049
Tetraenoics	0	0

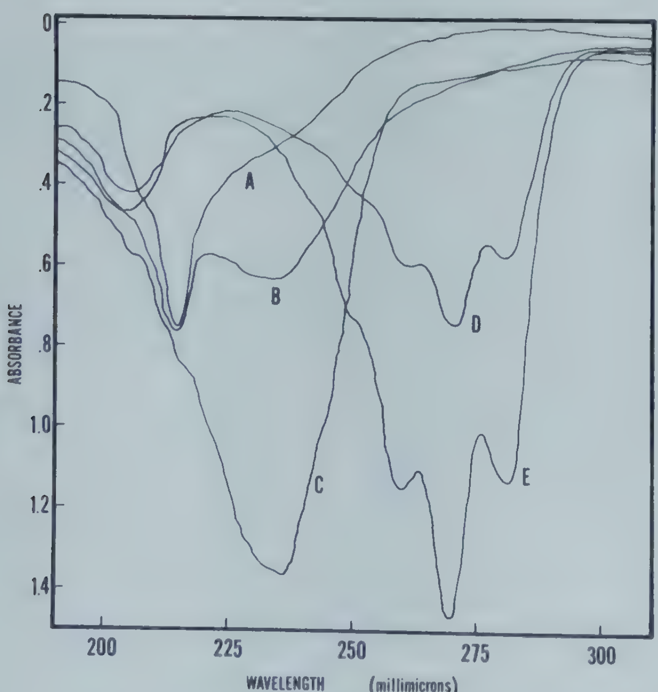

FIG. 2 Ultraviolet spectra of the crude oils from soybean, cottonseed, *C. foetidissima, C. digitata* and *A. undulata* in cyclohexane: A—soybean oil (0.051% w/v); B—cottonseed oil (0.054% w/v); C—*C. foetidissima* oil (0.051% w/v); D—*C. digitata* oil (0.0026% w/v); E—*A. undulata* oil (0.0025% w/v).

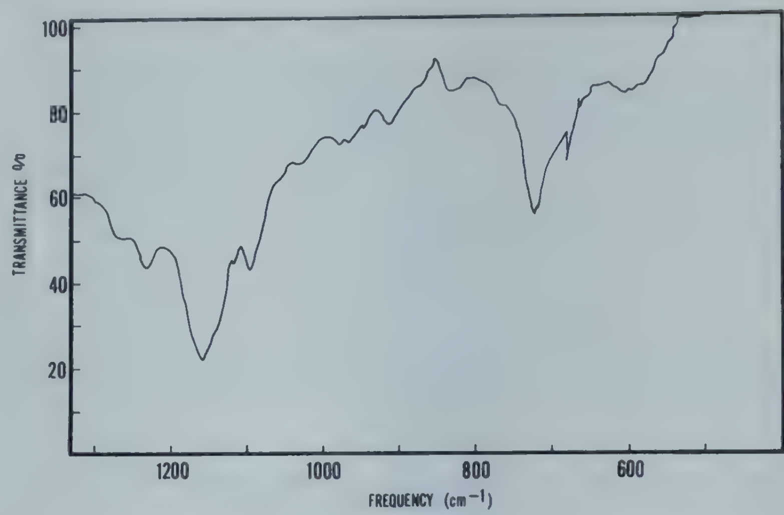

FIG. 3. Infrared spectrum of crude *C. foetidissima* oil in carbon disulfide (2.01% w/v).

Oleic, palmitic and stearic acids were present at levels of 22, 12 and 3.5%, respectively. The wide range of variation found for these fatty acids suggests the possibility of modification through plant breeding.

A comparison of the UV spectra of *C. foetidissima,* cottonseed and soybean oils for their content of conjugated dienoic fatty acids is shown in Figure 2. The level found in *C. foetidissima* oil was 2.3%, ca. 3 times that (0.8%) found in the sample of cottonseed oil and ca. 8 times the level (0.3%) found in soybean oil. This level, however, is similar to amounts found in some crude edible oils.

A comparison with oils of other xerophytic cucurbits for the quantity of conjugated trienoic fatty acids is also presented in Figure 2. The average level of these acids was found to be 0.03% in *C. foetidissima* whereas the levels in *Cucurbita digitata* and *Apodanthera undulata* were 8.6 and 17% of the total fatty acid content. Punicic acid, 9,11, 13-c,t,c-octadecatrienoic acid, has been reported in these 2 species at much higher levels (17 and 30%, respectively), suggesting wide variation of this fatty acid in *Cucurbita* species (11,12).

The levels of conjugated fatty acids found in *C. foetidissima* oil have no important effect on its possible use as an edible oil. They are not only low but equivalent to levels found in other oils of edible quality. Smith et al. (13), for example, report levels of total conjugated fatty acids ranging from 0.31 to 1.39% for 5 different samples of margarines and from 0.62 to 0.74% for 5 different samples of butter.

The infrared spectrum of *C. foetidissima* oil (Fig. 3) resembles closely those of cottonseed, corn and soybean oils. The absence of bands at 933 and 984 cm^{-1}, which are characteristic of *cis, trans, cis* conjugated linkages, reflects the negligible level of punicic acid (0.03%) already reported.

The oil properties which have been discussed indicate that this plant could become an important oilseed crop for arid land agriculture. Additional technical and agronomic studies are being made to determine the potential for domestication of this species.

ACKNOWLEDGMENT

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REFERENCES

1. Bemis, W.P., J.W. Berry, C.W. Weber and T.W. Whitaker, HortSci. 13:235 (1978).
2. Bemis, W.P., L.C. Curtis, C.W. Weber and J.W. Berry, Econ.

Bot. 32:87 (1978).
3. Jacks, T.J., T.P. Hensarling and L.Y. Yatsu, Ibid. 26: 135 (1972).
4. Berry, J.W., C.W. Weber, M.L. Dreher and W.P. Bemis, J. Food Sci. 41:465 (1976).
5. Scheerens, J.C., W.P. Bemis, M.L. Dreher and J.W. Berry, JAOCS 55:523 (1978).
6. Shahani, H.S., F.G. Dollear and K.S. Markley, Ibid. 28:90 (1951).
7. Official and Tentative Methods of the American Oil Chemists' Society, Vols. I and II, 3rd Edition, AOCS, Chicago, IL, 1970.
8. Zscheile, F.P., H.A. Nash, R.L. Henry and L.F. Green, Ind. Eng. Chem., Anal. Ed. 16:83 (1944).
9. Official Methods of Analysis of the Association of Official Agricultural Chemists, 10th Edition, AOAC, Washington, DC, Method 28.046.
10. Bailey, A.E., "Cottonseed and Cottonseed Products," Interscience Publishers, Inc., New York, 1948, p. 770.
11. Chisholm, J.J., and C.Y. Hopkins, JAOCS 43:390 (1966).
12. Bemis, W.P., M. Moran, J.W. Berry and A.J. Deutschman, Jr., Can. J. Chem. 45:2637 (1967).
13. Smith, J.M., W.L. Dunkley, A. Franke and T. Dairiki, JAOCS 55:257 (1978).
14. "Bailey's Industrial Oil and Fat Products," 3rd Edition, edited by D. Swern, Publishers, Ind. Div. of John Wiley and Sons, Inc., New York, 1964.

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Detection of Reesterified Oils: Determination of Fatty Acids at Position-2 in the Glycerides of Oils

D. GEGIOU and **M. GEORGOULI,** General Chemical State Laboratory, Research Department, 16 An. Tsoha Street, Athens 602, Greece

ABSTRACT

The IUPAC method II.D.27 for detection of reesterified oils by analysis of fatty acids at position-2 in fat and oil glyceride is reinvestigated and the effect of storage and different refining processes on the glyceride structure of genuine olive oil and olive-residue oil is studied. The method is unsuitable for oils neutralized by steam distillation because of changes in the triglyceride structure. An improved procedure is proposed in order to minimize erroneous conclusions for several other analyses.

INTRODUCTION

Reesterified oils are produced by esterification of glycerol and distilled fatty acids or by direct reesterification of oils with high percentages of free fatty acids. For the production of reesterified oils, olive oil or olive-residue oil often is used. The products thus obtained have an identical fatty acid composition to olive oil (fatty acid composition of olive oil and olive-residue oil is practically identical). Since reesterified oils are inexpensive, adulteration of the expensive olive oil or even the less expensive olive-residue oil with these oils is attractive.

Olive oil or olive-residue oil and reesterified oils can be characterized by their triglyceride structures. Saturated fatty acids, i.e., palmitic and stearic acids, are esterified almost exclusively at the combined 1,3-positions of olive oil and olive-residue oil triglycerides, as shown with many other triglycerides of vegetable origin (1,2). However, in reesterified oils, fatty acids have a statistical distribution on the 3-positions of glycerol. Determination of palmitic acid at position-2 of the glycerides of olive oil and olive-residue oil has been used for detecting adulteration by reesterified oils in these products. Published procedures include selective enzymatic hydrolysis by mammalian pancreatic lipase, separation of hydrolytic products by thin layer chromatography (TLC), conversion of isolated 2-monoglycerides to methyl esters, and their fatty acid analyses by gas liquid chromatography (GLC) (3,4).

In this study, a reinvestigation of IUPAC method II.D.27 (5) for the determination of fatty acids at position-2 in the glycerides of oils and fats is undertaken. The method has been provisionally agreed upon by the Codex Alimentarius Committee on Fats and Oils to be forwarded for assessment to the Codex Committee on Methods of Analysis and Sampling (6), and has been adopted by the EEC (7). Furthermore, the effect of storage and different refining processes on the glyceride structure of genuine olive oil and olive-residue oil and, consequently, on the results obtained by this method has been studied and an improved procedure is proposed in order to minimize erroneous conclusions for several analyses.

EXPERIMENTAL PROCEDURES

Samples

Samples of genuine virgin olive oil and olive-residue oil were collected from the main oil producing areas of Greece. Industrially processed samples of these oils were supplied by Elais Co., Athens, Greece, and by Avea Co., Crete, Greece.

MATERIALS AND METHODS

The determination of fatty acids at position-2 of glycerides was performed according to IUPAC method II.D.27 (5), except where indicated. Fatty acid methyl esters were prepared according to AOCS method (8) (BF_3 + methanol). Pancreatic lipase was from Sigma Chemical Co., USA. The chromatographic plates were from Macherey-Nagel & Co. (SIL G 50,UV 254). Agitation during hydrolysis was performed either with a magnetic stirrer or with a dental amalgamator. GLC: Varian 2800 or Tracor 550 with FID detectors. Columns: DEGS 15% on Chromosorb AW, 100-120 mesh, and EGGS 10% on Gas Chrom Q, 100-120 mesh, both 1.80 × 3 mm id of stainless steel.

Passage over kieselgel: A chloroform solution of oil (4 g in 66 ml) was percolated through a column (33 × 1.8 cm) with kieselgel 60, 70-230 mesh, (Merck, 30 g) at a rate of ca. 60 drops per min. The triglycerides were eluted with benzene (25 ml) at the same rate. The solvent was removed in a rotatory evaporator under nitrogen.

RESULTS AND DISCUSSION

The percentage of palmitic acid at position-2 in the glycerides of 62 samples of genuine virgin olive oil from the main

TABLE I

Percentage of Palmitic Acid at Position-2 in the Glycerides of Genuine Virgin Olive Oil (n = 62) and Genuine Olive-residue Oil (n = 30) from Greece

	Minimum	Maximum	Mean	Palmitic acid (%)	
				No. > 1.5[a]	No. > 2.0[b]
Olive Oil (48 samples, acidity to 3.3%)	0.6	2.7	1.5	20	3
				No. > 1.8[a]	No. > 2.0[b]
Olive oil (14 samples, acidity = 3.7-9.2%)	1.4	3.6	2.6	9	9
				No. > 2.2[a]	No. > 2.0[b]
Olive-residue oil (30 samples, crude or neutralized)	1.2	4.0	2.4	12	18

[a]Limits proposed by the International Olive Oil Council (IOOC), expressed as saturated acids (9), i.e., palmitic + stearic acids. The percentage of stearic acid is too small to be measured accurately.

[b]Limits proposed by the European Economic Community (EEC).

oil-producing areas of Greece (years of production: 1975, 1976) was determined. Among the 62 samples, 48 were edible olive oil samples, i.e., having free acidity up to 3.3% (as oleic acid), and 14 had free acidity 3.7-9.2% which would be consumed after refining. The determination was also performed on 30 samples of genuine olive-residue oil from Crete and Corfu produced in 1975, 1976 and 1977. The samples of olive-residue oil were either crude or neutralized by alkali, soda or distillation. The results are summarized in Table I.

From the results of Table I, it is seen that the limit set by the EEC (7) may be considered adequate for edible virgin olive oil and that the limit proposed by the IOOC for this oil is too low. More interesting is the observation that for oils with higher than 3% acidities, which, according to II.D.27 have to be neutralized with alkali in the presence of a solvent prior to hydrolysis, and for oils which have been neutralized in industry the limits appear too low. According to this observation, we have investigated the effect of the neutralization steps described by II.D.27 on the glyceride structure of oils in order to evaluate the method.

IUPAC method II.D.27 includes the following steps: (a) neutralization with alkali in the presence of a solvent for oils having acidities higher than 3%; (b) passage through a column with activated alumina to purify the samples and also to neutralize oils having acidities up to 3%; (c) hydrolysis with pancreatic lipase; (d) isolation of the 2-monoglycerides by TLC; (e) conversion of the 2-monoglycerides to methyl esters; (f) GLC analysis of the methyl esters; (g) expression of results as saturated acids at position-2 of glycerides.

Steps a and b

Several samples of olive oil and olive-residue oil with acidities ranging from 3.3-35.5% were neutralized with alkali according to step a, with soda, over alumina according to step b, and over kieselgel. Using the column with kieselgel as described in Experimental Procedures, oils with acidities up to 35% may be neutralized. The results shown in Table II indicate that steps a and b clearly affect the glyceride structure, probably because some hydrolysis of ester bonds followed by acyl migration is taking place. The effect is stronger with alkali, but alumina also results in higher percentages of palmitic acid in position-2 when compared to kieselgel. It has been reported that with alumina, some hydrolysis of triglycerides ester bonds may occur (10). In order to verify the effect of alkali, 3 samples were left with the neutralizing solution for 3 hr; the results indicate it may be preferable to achieve neutralization of oils prior to hydrolysis by kieselgel and eliminate both steps a and b.

Step c

Concerning enzymatic hydrolysis (step c), method II.D.27 states the tube containing the hydrolysis mixture should remain in a thermostat at 40 ± 0.5 C for 1 min (under agitation) after which it is removed and agitated for another

TABLE II

Effect of Neutralization Steps on the Percentage of Palmitic Acid in Position-2 in the Glycerides of Olive Oil and Olive-residue Oil

Sample	Acidity (%)	Alkali	Soda	Alumina	Alkali (3 hr)	Kieselgel
Olive oil	3.3	2.0	-	1.4	-	-
	4.4	3.4	2.6	2.6	-	1.8
	4.8	1.4	-	1.1	-	1.0
	5.5	2.9	2.3	1.5	-	1.2
	5.6	1.6	-	1.0	-	-
	5.9	2.8	2.1	1.4	-	-
	7.0	1.5	-	0.9	-	-
	8.0	2.1	-	1.1	-	-
	9.2	3.1	2.3	1.4	-	1.1
Olive-residue oil	16.6	3.9	-	1.8	8.0	1.4
	18.8	3.5	2.7	2.0	5.9	1.4
	19.1	2.6	-	1.6	5.7	1.2
	22.5	2.7	2.1	-	-	-
	35.5	4.0	3.4	-	-	-

2 min at room temperature, and then cooled under running water before 1 ml of 6 N hydrochloric acid is added to stop hydrolysis. We measured the temperature of the tube content after 0.5 and 1 min during the hydrolysis for 1 min in the thermostat and it was 32.5 and 37 C, respectively. Thus, the duration of hydrolysis is extended to more than 3 min, which is considered long, whereas the temperature for a fraction of a min only reaches 37 C. The hydrolysis conditions should be chosen to achieve deacylation as rapidly as possible and to minimize undesirable acyl migration in the partial glycerides. Optimal reaction conditions are considered, i.e., reaction temperatures 37-40 C and duration of hydrolysis < 90 sec (11). The Luddy et al. (3) semimicro procedure is recommended by which the tube and contents are first warmed at 40 C for 1 min without shaking, followed by vigorous shaking for 1 min at the same temperature.

Luddy et al. (3) report that the degree of hydrolysis with their procedure (enzyme/sample ratio is similar in the 2 methods) reaches 50 ± 5%. Using the II.D.27 procedure, we found that, by weighing the fatty acids isolated by TLC, the hydrolysis did not exceed 22%.

Step d

After hydrolysis is stopped with 6 N hydrochloric acid, the hydrolysis products are extracted with diethyl ether. According to step d, the 2-monoglycerides are then isolated by TLC with a developing solvent consisting of hexane/diethyl ether/formic acid, 70:30:1 (v/v/v). Under these conditions, the R_f of 2-monoglycerides is ca. 0.035, which is low. The amount of formic acid in the developing mixture should be increased to 2-3% in order to double the R_f value and avoid removal of components remaining on the baseline.

Step g

The IOOC has proposed to the Codex Committee on Fats and Oils that results be expressed as saturated acids in position-2 of glycerides (i.e., palmitic + stearic acids). Stearic acid in the fatty acids of olive oil does not exceed 2.5-3%, thus the percentage of stearic acid in position-2 is too small to be calculated accurately. We recommend that results be expressed as percent palmitic acid, which has been adopted already by the EEC (7).

Storage

We investigated the effect of storage on the glyceride structure—especially of oils with high acidities resulting in considerable partial glyceride content.

Samples of crude olive-residue oil, with acidities ranging from 10.2-35.5% as oleic acid stored for 1-2 years, have shown considerable increases in the percentage of palmitic acid at position-2 of their glycerides when examined by method II.D.27. On the contrary, 2 samples of olive oil with acidities 0.9% and 2.1% have shown no alteration.

Since the structure of partial glycerides is expected to be altered with time, palmitic acid at position-2 is also determined in the pure triglycerides of the oils. Columns with kieselgel are used for neutralization purposes, but in this case, also for isolating the pure triglycerides of the oils (12). The results of Table III suggest that the enzymatic hydrolysis should be performed on the pure triglycerides, at least for oils containing considerable percentages of partial glycerides in which the structure is altered during storage. This is a second reason that chromatography over kieselgel is preferred versus neutralization with alkali and/or over alumina of oils prior to hydrolysis.

Refining

In order to compare possible alterations caused by different refining procedures, palmitic acid in position-2 of glycerides has been determined according to II.D.27 and according to the improved procedure proposed here, i.e., neutralization and isolation of pure triglycerides over kieselgel in 3 crude olive-residue oils (acidities 17.5, 21.4 and 24.5%). The determination was repeated in the same oils after one part of each of them was neutralized industrially with soda and the other part by steam distillation, and in their final

TABLE III

Effect of Storage on Percentage of Palmitic Acid at Position-2 in the Glycerides of Olive-residue oil and Virgin Olive Oil

	Acidity (% oleic acid)	According to II.D.27 (IUPAC)	With neutralization and isolation of pure glycerides over kieselgel
Olive-residue oil produced in 1975 and examined in:			
1976	10.2	2.2	
	18.9	1.8	
1978	15	3.1	1.5
	26	4.4	1.3
Olive-residue oil produced in 1976 and examined in:			
1977	22.5	2.7	
	18.8	3.5	
	16.6	3.9	
	35.5	4.0	
1978	24.6	4.5	1.4
	24.2	5.8	1.3
	18.6	5.4	1.8
	38.5	5.0	1.7
Virgin olive oil produced in 1976 and examined in:			
1977	2.1	1.5	
	0.9	1.1	
1978	2.2	1.6	1.4
	0.9	1.2	1.1

TABLE IV

Effect of Different Refining Processes on the Percentage of Palmitic Acid at Position-2 in the Glycerides of Olive-residue Oil and Olive Oil

Sample	Crude	Neutralized with soda	Refined	Neutralized by distillation	Refined
Olive-residue oil I	4.2[a]	3.1[a]	3.3[a]	5.4[a]	5.7[a]
	2.0[b]	1.1[b]	1.2[b]	4.0[b]	3.9[b]
Olive-residue oil II	2.6[a]	2.9[a]	2.8[a]	4.2[a]	4.4[a]
	1.8[b]	2.0[b]	2.0[b]	3.7[b]	3.8[b]
Olive-residue oil III	2.9[a]	2.9[a]	2.8[a]	5.8[a]	5.7[a]
	1.5[b]	1.8[b]	1.9[b]	4.2[b]	4.2[b]
		Neutralized with alkali			
Olive oil I	2.0[a]	1.4[a]	1.4[a]	-	-
Olive oil II	1.8[a]	1.6[a]	1.6[a]	-	-
	0.9[b]	0.9[b]	1.1[b]	-	-

[a]According to IUPAC method II.D.27.

[b]According to the proposed improved procedure, i.e., neutralization and/or isolation of pure triglycerides over kieselgel.

refined products. Moreover, determinations have been performed on 2 samples of olive oil (acidities 3.5 and 3.8%) before refining, after neutralization with alkali and on the final refined products. The results are shown in Table IV. Again the need to eliminate steps a and b of method II.D.27 and replacement by kieselgel column chromatography are indicated. Furthermore, by comparing results obtained by the 2 different industrial neutralization procedures, it is apparent that steam distillation affects the triglyceride structure of the oils to a considerable extent. Steam distillation had been previously reported to affect the glyceride structure of oils (13). Thus, the method is found to be inapplicable to oils neutralized by steam distillation. Therefore the part of the footnote is necessary which was proposed by an IUPAC observer at the Tenth Session of the Coeex Committee on Fats and Oils (14): and reads: "Certain refining processes may affect the glyceride structure of the oil and lead to the level of palmitic acid in position-2 of genuine oils that have not been subjected to reesterification being above the maxima given." This statement is to be included in our proposed procedure. Otherwise, refining processes do not affect the results obtained to a detectable extent.

REFERENCES

1. Mattson, F.H. and E.S. Lutton, J. Biol. Chem. 233:868 (1958).
2. Mattson, F.H. and R.A. Volpenheim, J. Biol. Chem. 236:1891 (1961).
3. Luddy, F.E., R.A. Barford, S.F. Herb, P. Magidman and R.W. Riemenschneider, JAOCS 41:693 (1964).
4. Privett, O.S. and L.J. Nutter, Lipids 2:149 (1967).
5. "Standard Methods for the Analysis of Oils, Fats and Soaps," IUPAC 6th edition.
6. Codex Alimentarius Commission, Report of the Ninth Session of the Codex Committee on Fats and Oils, Alinorm 78/17.
7. J. Off. Com. Eur., 20e année, N° L 128/16 (1977).
8. "Sampling and Analysis of Commercial Fats and Oils," AOCS, Method Ce 2-66, revised 1969.
9. Codex Alimentarius Commission, Report of the Tenth Session of the Codex Committee on Fats and Oils, Alinorm 79/17.
10. Litchfield, C., "Analysis of Triglycerides," Academic Press, New York and London, 1972, p. 161.
11. Litchfield, C., Ibid. p. 175.
12. Gunstone, F.D., R.J. Hamilton and M. Ilyas Qureshi, J. Chem. Soc. (London) 319 (1965).
13. Taponeco, G. and G. Ghimenti, Riv. Ital. Sost. Grasse 50:5 (1973).
14. Joint FAO/WHO Standard Programme, Tenth Session of the Codex Committee on Fats and Oils, CX/FO 78/7.

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Soaps, Detergents, and Cosmetics

Effect of Alkyl-2-pyrrolidone-5-carboxylate As an Additive on Emulsion Stability

H. SHIMADA, K. YAMAZAKI, M. UENO and **K. MEGURO,** Department of Chemistry, Faculty of Science, Science University of Tokyo, Kagurazaka 1-3, Shinjuku-ku Tokyo, Japan 162

ABSTRACT

Alkyl-2-pyrrolidone-5-carboxylates (R-PCA), the derivatives of higher alcohols, were prepared and their properties as emulsion stabilizers were estimated using surface tension meter and Multiple Drop methods in the presence of sodium dodecyl sulfate (SDS) and were compared to those of another system containing a series of the corresponding alcohols as stabilizers. The R-PCA system markedly reduced the interfacial tension compared to a higher alcohol system, and the interfacial tensions for both R-PCA and higher alcohol systems decreased with the increase of carbon chain length. The stability of oil drops increased greatly by addition of R-PCA compared to higher alcohol system. In the higher alcohol system, the stability of oil drop showed the minimum at carbon 12. In the R-PCA system, the stability of oil drops increased greatly with the increase of carbon chain length. A series of R-PCA was found to be useful as emulsion stabilizers.

INTRODUCTION

Some higher fatty alcohols have been reported to be very useful as stabilizers of emulsions (1-9). Schulman and Cockbain (2) have found that the stability of Nujol-in-water emulsions increases markedly by an addition of cetyl alcohol, which results from a formation of intermolecular complexes with emulsifiers at oil-water interface. Davies and Mayers (3) also have reported that simultaneous adsorption of sodium dodecyl sulfate and cetyl alcohol to benzene-water interface results in a very high interfacial viscosity and then forms more stable emulsions compared to the system including only sodium dodecyl sulfate as an emulsifier. The reason emulsions become more stable by adding a small amount of higher fatty alcohols has so far been explained by more stable complex formations between alcohols and emulsifiers.

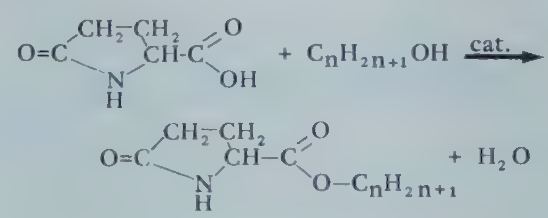
We are interested in some effects of alcohol derivatives of DL-2-pyrrolidone-5-carboxylic acid (PCA) as emulsion stabilizers, these being regarded as some of the most important natural moisturizing factors (10-13). Some workers have reported that a superior moisturizing effect is contributed to the properties of cosmetic emulsions by an addition of a small amount of stabilizers such as PCA salts or their derivatives (such as higher fatty alcohol esters of PCA) (14-17). However, their studies have not been made systematically for higher alcohols and their PCA derivatives. A higher alcohol ester of PCA can be expected to have better properties as an emulsion stabilizer than the higher alcohol.

In this study, PCA esters of alcohols with chain length of 8-16 carbon atoms are prepared. Their effects as emulsion stabilizers for the toluene-in-water emulsion system are discussed on the basis of experimental results obtained by Wilhelmy-type surface tension meter and Multiple Drop methods. These are compared to results for the corresponding higher alcohols.

EXPERIMENTAL PROCEDURES

Materials

Alkyl-2-pyrrolidone-5-carboxylate (R-PCA) was prepared by usual esterification of DL-2-pyrrolidone-5-carboxylic acid with corresponding higher alcohol in a toluene solvent using *p*-toluene sulfonic acid as a catalyst.



Water formed as a byproduct was separated as the toluene azeotrope and measured volumetrically. The endpoint of reaction was found from the amount of water as byproduct. The unreacted PCA and a trace of catalyst were removed from toluene solution by adding ca. 1 M (10 wt%) sodium carbonate solution. After the toluene was distilled, the reaction product was purified by crystallization 3 times from n-hexane. The purity of the sample was confirmed by IR and elemental analysis.

Sodium dodecyl sulfate (SDS) for biochemical use (Wako Pure Chemical Industries, Ltd., Tokyo) was extracted with petroleum ether for 100 hr to remove traces of alcohols, then purified by crystallization 3 times from ethanol and carefully dried in vacuo for 60 hr before use. The purity of the sample was confirmed by the absence of a minimum in the surface tension vs concentration curve. The CMC value of SDS, determined by conductivity method in water at 25 C, was 8.00 mmol/ℓ, in good agreement with the literature value at 25 C (18).

Higher fatty alcohols were the extra pure grade products (higher than 99%, Wako Pure Chemical Industries, Ltd., Tokyo) used without further purification.

Toluene was GR and used without further purification. The water used was passed through a Milli-Q Water Purification System (Nihon Millipore Co., Tokyo) until its specific conductivity fell below $10^{-7}\,\Omega^{-1}\,cm^{-1}$.

Apparatus and Procedure

The interfacial tension was measured with a Wilhelmy-type surface tension meter (Shimadzu Surface Tensiometer ST-1). The oil-soluble substance (higher alcohol or R-PCA) was dissolved in toluene as the oil phase, whereas SDS was dissolved in the water phase. The interfacial tensions at the toluene (oil-soluble substance containing)/water (SDS containing) interface were measured at various concentrations of the oil-soluble substances at 25 C. The concentration of SDS in the water phase was fixed at 8.00 mmol/ℓ

TABLE I

Characteristics of Additives Used

Chemical composition	Abbr.	Carbon chain length	Melting point (C)
Octyl-2-pyrrolidone-5-carboxylate	Oc-PCA	8	40
Decyl-2-pyrrolidone-5-carboxylate	De-PCA	10	52
Dodecyl-2-pyrrolidone-5-carboxylate	La-PCA	12	60
Tetradecyl-2-pyrrolidone-5-carboxylate	My-PCA	14	68
Hexadecyl-2-pyrrolidone-5-carboxylate	Ce-PCA	16	73
Octyl alcohol	Oc-OH	8	-15
Decyl alcohol	De-OH	10	7
Dodecyl alcohol	La-OH	12	24
Tetradecyl alcohol	My-OH	14	38
Hexadecyl alcohol	Ce-OH	16	50

near the cmc to obtain uniform and stable droplets for the measurements of the stability of the oil droplets (19). These measurements were started 10 min after initial contact between the 2 phases, since the equilibria at these interfaces were reached after 5 min (20).

To measure the stability or persistence of oil drops at the oil-water interface, an apparatus shown in Figure 1 similar to that of Cockbain and McRoberts was used (19).

The apparatus consists of vessel A (diameter 21 cm) in which the oil-water interface is made, a thermostated water jacket B and tip C to form the oil drop. Tip C has 3 parts: a syringe (capacity 2 ml), a syringe needle with flattened tip and a micrometer which is modified to push the syringe piston and then measure the distance travelled by the piston. As Cockbain et al. (19) found, the stabiltiy of the drops was hardly affected by volume in the range 0.0005-0.01 ml. Therefore, the size of the drop was maintained at ca. 2×10^{-3} ml by changing the needle size. The distance from the tip of C up to the interface was ca. 2 cm. After detaching a drop from the top of C, it ascends in vessel A and then comes into contact with a planar oil-water interface and finally coalesces with time. The time of coalescence or lifetime of the drops until 30 oil drops coalesce at the interface was measured at 25 C using a stop watch. When the last drop comes into contact with the oil-water interface, the measurement of lifetime was started. The formation of the 30 oil drops required 30 sec, but the time for setting of the oil drops did not affect the experimental data.

The experiment was made with the toluene-water system containing various concentrations of the oil-soluble substances in the toluene phase as additives and 8 mmol/ℓ of SDS in the water phase as an emulsifier. When the number of drops remaining without coalescence (N) was plotted in logarithmic scale against time (t), most plots were found to be linear in the range of part AB on the curve, as shown in Figure 2, in agreement with the results obtained by Cockbain et al. (19).

In general, the overall stability of droplet can be evaluated by the time required for half the droplets to coalesce (t 1/2) (19, 21-22); these half-life time values were used in order to estimate the stability of oil drops in this experiment.

RESULTS AND DISCUSSION

In general, it is necessary to satisfy the following requirements in order to make a stable emulsion: (a) small differences in density between the dispersed and continuous phases; (b) high viscosity of the continuous phase; (c) small interfacial tension between the dispersed and continuous phases; (d) expanded electrical double layer existing at the oil-water interface; and (e) mechanical strength in the adsorbed layer at the oil-water interface. In the presence of additives (oil-soluble substances), the most important factors affecting substantial emulsion stability seem to be interfacial tension and mechanical strength of the interfacial film. Therefore, coalescence of droplets and interfacial tension may be the most important factors in order to discuss the stability of emulsions.

Figure 3 shows the effects of addition of various alcohols and the PCA esters of corresponding alcohols as the oil-soluble substances on the interfacial tensions at the toluene/SDS solution. In both cases, the interfacial tensions decreased with increasing concentrations of alcohols or R-PCA, dissolved in the oil phase. The effectiveness in reducing the interfacial tensions in the R-PCA was larger than for the alcohols over the entire concentration range, as shown in Figure 3. Notably, the interfacial tension could decrease to as low as 2 dyn/cm even in a dilute toluene

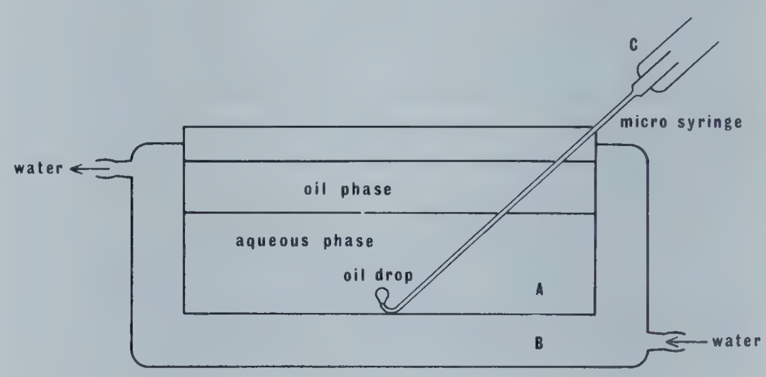


FIG. 1. Apparatus for determining the lifetime of oil drops.

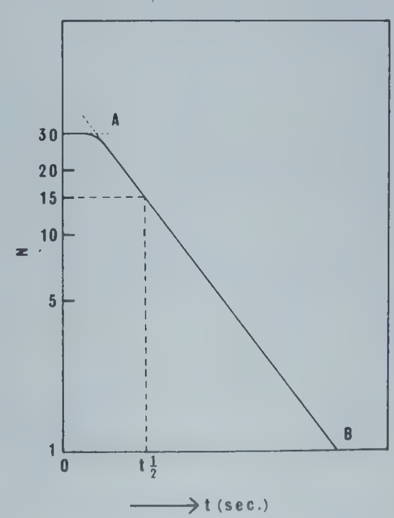


FIG. 2. Typical relationship between N (in logarithmic scale) and t.

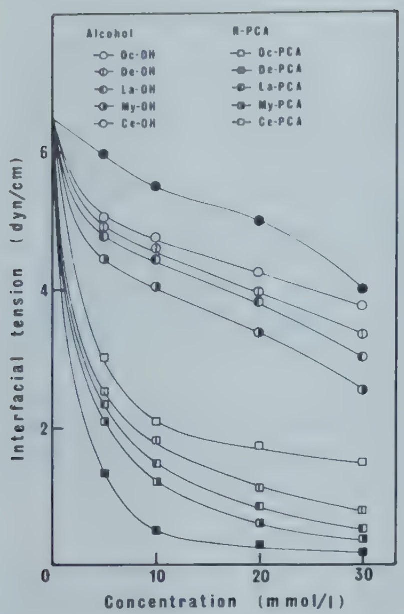


FIG. 3. Effect of oil-soluble substances on the interfacial tension at toluene/SDS soln interface. Water phase: 8 mmol/ℓ SDS soln.

solution of R-PCA. This suggests that SDS molecules interact with R-PCA molecules more strongly than higher alcohol molecules, probably because of the difference of the ion-dipole interactions between SDS and additives. To know the influence of the hydrophobic group in the additives (oil-soluble substances) on the interfacial tension, the interfacial tensions for 2 series of higher alcohols and R-PCA were plotted against the carbon numbers of the corresponding alcohols as shown in Figure 4.

Both these interfacial tensions decreased with increasing chain length except for the alcohols, where a minimum was observed at C_{14}; that is, the interaction of additives with SDS is found to increase with the increase in the effect of hydrophobic groups in the additives.

Figures 5 and 6 show the effect of additives (higher alcohol and R-PCA) on the stability of oil drop at the toluene/SDS solution interface. As shown in Figure 5 for higher alcohols, the stability of oil drop increased with additive concentration, but decreased at its high concentration. This result suggests that the surface of oil drop is completely occupied by the alcohol molecules, and then the adsorbed amount of SDS at the oil-water interface decreases. As shown in Figure 6 for R-PCA, the stability of an oil drop was remarkably increased above a certain concentration and the oil drops hardly coalesced. Oc-PCA showed behavior similar to the higher alcohol, but the effect of Oc-PCA on the stability of oil drop was larger than that of the higher alcohols. Therefore, it is recognized that oil drop can be markedly stabilized by the R-PCA system compared to the higher alcohol system at all concentrations. Furthermore, apparently SDS interacts with R-PCA more strongly than higher alcohol. It is concluded that a pyrrolidone ring plays an important role in making the oil drop stable. This also suggests that pyrrolidone rings have some ability to capture SDS molecules from aqueous solution into the surface of oil droplets. Accordingly, molecular association at an oil-water interface between SDS and R-PCA molecules can be considered to be different from those of higher alcohols.

Figures 7 and 8 show the effect of hydrocarbon chain lengths of higher alcohols and R-PCA, respectively, on the stability of oil droplets at the toluene/ aqueous SDS solution interface. The stability of oil droplets for the higher alcohol systems decreased with increased hydrocarbon chain length of carbon atoms 8-12, then increased with increased chain length from 12 to 16 and showed just a minimum at C_{12} corresponding to lauryl alcohol as shown in Figure 7.

This suggests that the polar groups in alcohols with carbon chain length below C_{12} interact with the ionic groups of SDS more effectively than with the hydrocarbon chain parts. The increase in the stability of the system using alcohols above C_{12} can be considered to result from stronger chain-chain interaction between alcohol and SDS. The minimum in Figure 7 appears at carbon 12 just where both effects of polar and hydrocarbon groups are compensated. In the R-PCA systems, the stability of oil drops depended on the hydrocarbon chain length and gradually increased with increasing chain length at all concentrations, then markedly increased above a certain carbon chain length, as shown in Figure 8. This apparently results from the stronger interaction between hydrocarbon chains of R-PCA and SDS.

According to Dervichian (23) it has been reported that the van der Waals forces between the hydrocarbon chains of emulsifier molecules primarily result in the regular arrangement of the molecules in mixed monolayers and also that emulsifier molecules cannot associate easily in surfactants with hydrocarbon chain below 8 C atoms. From these suggestions and experimental findings, it may be concluded that a molecular association between SDS and additives is dependent of the van der Waals forces between their hydrocarbon chains or on the ion-dipole interactions

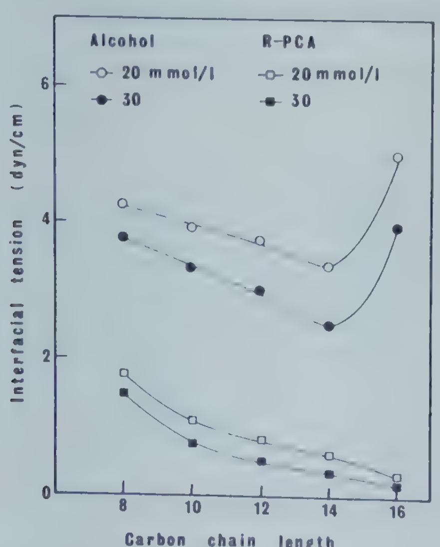


FIG. 4. Effect of hydrophobic group in oil-soluble substances on the interfacial tension at toluene/SDS soln interface.

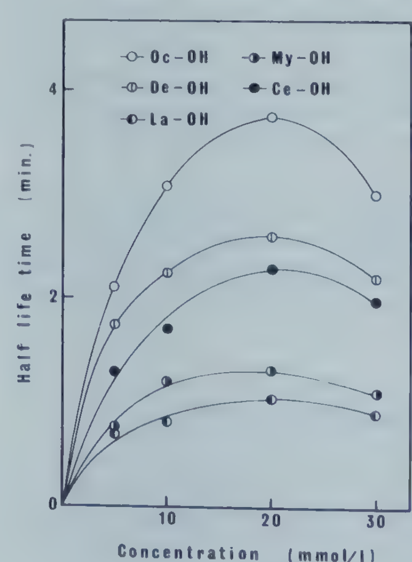


FIG. 5. Effect of higher alcohols on the stability of oil drop at toluene/SDS soln interface. Water phase: 8 mmol/ℓ SDS soln.

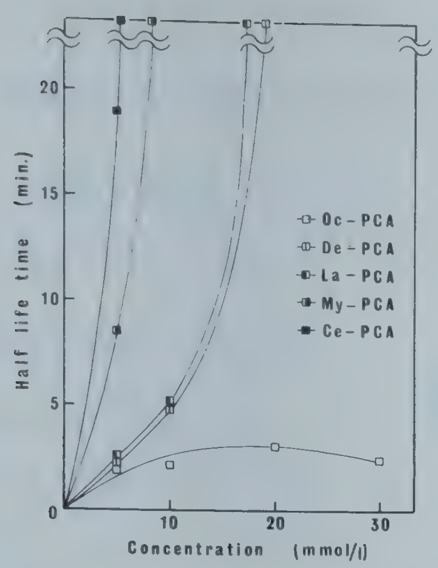


FIG. 6. Effect of R-PCA on the stability of oil drop at toluene/SDS soln interface. Water phase; 8 mmol/ℓ SDS soln.

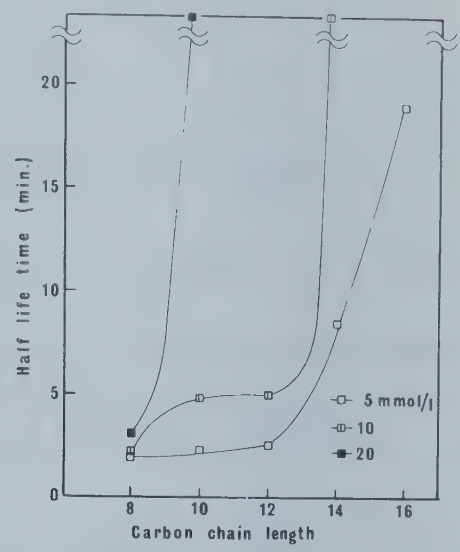


FIG. 8. Effect of hydrophobic group in R-PCA on the stability of oil drop at toluene/SDS soln interface.

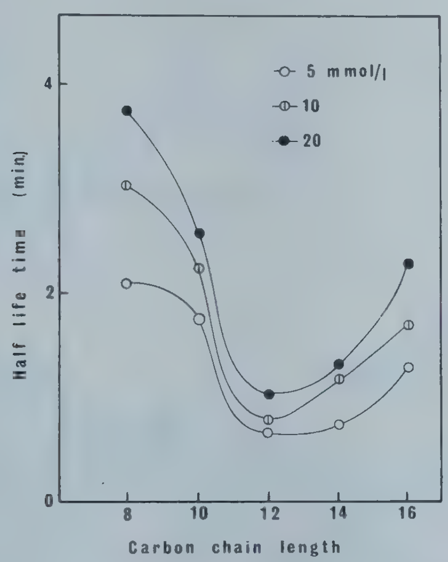


FIG. 7. Effect of hydrophobic group in higher alcohols on the stability of oil drop at toluene/SDS soln interface.

between their head groups, and also that the mechanically stronger interfacial film is formed because of stronger interaction between SDS and additive molecules.

In general, the ionic character of SDS has been reported to create an electrical double layer at the oil-water interface in addition to the film barrier, thus inhibiting coalescence. Therefore, it is considered that the stability of an oil drop depends on the adsorbed amount of SDS available for inhibition of coalescence. It is also recognized that a condensed mixed monolayer of SDS and additives on the surface of emulsion drops is necessary for the stability of the oil drop, and the instability of the oil drop is caused by an incomplete oil surface coverage. Many investigators have reported that higher alcohols form a stable complex with SDS at the oil-water interface (1-9), but it is expected that as SDS molecules are not sufficiently adsorbed at the oil-water interface because of their electric repulsion, even if SDS-alcohol complex is formed, a stable 2-dimensional complex is not formed. When R-PCA are used as additives in the oil phase, the stronger interfacial film is considered to be formed because parts of a charge on SDS will be neutralized by the dipole of R-PCA, and the adsorbed amount of SDS to the oil-water interface will be increased.

REFERENCES

1. Schulman, J.H., and E.K. Rideal, Proc. R. Soc. (London) 46: 122B (1937).
2. Schulman, J.H., and E.G. Cockbain, Trans. Farady Soc. 36:651 (1940).
3. Davies, J.T. and G.R.A. Mayers, Ibid. 56:691 (1960).
4. Kung, H.C., and E.D. Goddard, J. Phys. Chem. 67:1965 (1963).
5. Carless, J.E., and G. Hallworth, J. Colloid Sci. 26:75 (1968).
6. Kung, H.C. and E.D. Goddard, J. Phys. Chem. 68:3465 (1964).
7. Vold, R.D., and K.L. Mittal, J. Colloid Interface Sci. 38:451 (1972).
8. Fukushima, S., M. Takahashi and M. Yamaguchi, Ibid. 57:201 (1976).
9. Elworthy, P.H., A.T. Florence and J.A. Rogers, Ibid. 35:34 (1971).
10. Laden, K., Amer Perfum. Cosmet. 82:77 (1967).
11. Laden, K., and R. Spitzer, J. Soc. Cosmet. Chem. 13:263 (1962).
12. Laden, K., and R. Spitzer, Ibid. 18:351 (1967).
13. Laden, K., and R. Morrow, Ibid. 21:417 (1970).
14. Yamagishi, M., Japan Patent Tokko 45-15392 (1970).
15. Ichikawa, T., Japan Patent Tokkai 50-22008 (1975).
16. Sumida, S., Japan Patent Tokko 51-35474 (1976).
17. Yunber, F., Japan Patent Tokkai 52-122637 (1977).
18. Hayase, K., and S. Hayano, Bull. Chem. Soc. (Japan) 50:83 (1977).
19. Cockbain, E.G., and T.S. McRoberts, J. Colloid Sci. 8:440 (1953).
20. Ogino, K., and M. Ota, Bul. Chem. Soc. (Japan) 49:1187 (1976).
21. Nielsen, L.E., R. Wall, and G. Adams, J. Colloid Sci. 13:441 (1958).
22. Edge, R.M., and M. Greaves, Inst. Chem. Eng. Symp. Series 26:63 (1967).
23. Dervichian, D.G., in "Surface Phenomena in Chemistry and Biology," edited by P. Sherman, Academic Press, New York, 1968, pp.82-89.

[Received October 18, 1979]

Industry News

Interox opens new plant

Interox America has begun production at a new plant in Deer Park, TX, that can produce up to 35 million tons per year of the firm's sodium percarbonate product, a free-flowing granular solid.

The firm expects major usage to be in heavy duty detergents and dry bleaches. □

Ethyl Corporation expanding facilities

Ethyl Corporation has announced plans for a $50 million expansion of its alpha-olefin facilities in Houston, TX.

The entire project is expected to be completed within two years and will result in a 50% increase in the firm's alpha-olefin capacity, Ethyl said. □

Meetings

SCC call for papers

"Reduction of Art to Science" has been selected as the theme for the 1981 Society of Cosmetic Chemists Scientific Seminar, to be held May 21–22, 1981, at the Hyatt Regency Hotel in Washington, DC.

Papers are being solicited on new technology, particularly in the areas of emulsification, viscosity control, dispersion and suspension of solids, and stabilization of products. In addition, papers on in vitro testing methods for product efficacy or cosmetic attributes are being solicited. Authors of pertinent papers are invited to submit 150-word abstracts to program cochairmen, Paul Thau and William E. Snyder, c/o Society of Cosmetic Chemists, 1995 Broadway, Suite 1701, New York, NY 10023. □

Abstracts

MATHEMATICAL RELATIONS CORRELATING PLURONICS PROPERTIES WITH THEIR CHEMICAL STRUCTURE. J. Szymanowski. *Fette, Seifen, Anstrichm.* *82*(2), 59-63 (1980). Statistically valid equations correlating properties of ethylene and propylene oxides block co-polymers of Pluronic type with molecular mass of polyoxybutylene chain, the content of ethylene oxide and concentration are given. Properties considered are density, viscosity, melting point, cloud point, surface and interfacial tension, foaming, wetting and washing ability.

THE DETERMINATION OF THE EFFECTIVE HLB OF NONIONIC SURFACTANTS BY A PHENOL TITRATION I: RELATION TO CLOUD POINT. L. Marszall. *Fette, Seifen, Anstrichm.* *82*(1), 40-5(1980). The qualitative similarities in two methods of the HLB (Hydrophile-Lipophile Balance) determination based on the cloud point and so-called phenol index measurements are

Continued on page 708A.

demonstrated. The effects of polyoxyethylene chain length, surfactant concentration and the presence of additives on the cloud point and phenol index values have been compared. The results are interpreted as the change of the effective HLB of polyoxyethylene nonionic surfactants.

SURFACTANTS AND POLYMERS–MATERIALS FOR THE TERTIARY PETROLEUM EXTRACTION IN HIGHLY SALINE SYSTEMS. M. Akstinat. *Tenside Deterg.* 17(1), 1-9 (1980). The importance and necessity of tertiary petroleum extraction methods is stressed. There are detailed discussions of the choice of polymers and surfactants, as well as various suitability criteria for viscous flooding media.

THE ANALYSIS OF CARBOXYMETHYLATED ETHOXYLATES. E. Kunkel. *Tenside Deterg.* 17(1), 10-2 (1980). Carboxymethylated ethoxylates can be analytically determined by means of thin-layer chromatography. After separation on the thin layer chromatography plate, the substance spots, dyed with Dragendorff reagent are quantitatively assessed through measurement of remission. As an alternative to thin-layer chromatography it is also possible to carry out a Dragendorff semi-micro precipitation with photometric evaluation of the bismuth content.

QUANTITATIVE DETERMINATION OF NON-IONIC SURFACTANTS IN WATER AND EFFLUENT BY ATOMIC ABSORPTION SPECTROSCOPY. J. Chlebicki and W. Garncarz. *Tenside Deterg.* 17(1), 13-7 (1980). Analytical methods for the determination of nonionic surfactants in natural waters are briefly reviewed. A new method for the determination of these compounds has been developed, in which advantage is taken of the precipitation of complex compounds on nonionic surfactants with molybdophosphoric acid. The acid is added in excess to a sample of pretreated water and the unreacted acid is subsequently determined by the atomic absorption spectraoscopic method. A calibration curve was obtained with oxyethylated nonylphenol as a standard.

CHEMICAL STRUCTURE AND SURFACE ACTIVITY. PART II. SYNTHESIS AND SURFACE PROPERTIES OF2-ALKYL-4-HYDROXYMETHYL-1,3-DIOXOLANES AT THE OIL-WATER INTERFACE. B. Burczyk and L. Weclas. *Tenside Deterg.* 17(1), 21-4 (1980). 2-Alkyl-4-hydroxymethyl-1,3-dioxolanes were synthesized by transacetalization of 2,2-dimethyl-1,3 dioxolane with straight chain aliphatic aldehydes and subsequent hydrolysis of the acetates obtained. The interfacial tension of these products in rape seed oil and paraffin oil was determined by the drop weight method. The surface activity of alkyldioxolanes increase in systems where the oil phase is less polar.

PRECIPITATION OF INSOLUBLE SALTS ON FABRICS LAUNDERED IN HARD WATER IN THE PRESENCE OF DIFFERENT ALKALIS AND BUILDERS. II. TWO-COMPONENT FORMULATIONS BASED ON SODIUM CARBONATE. S.V. Vaeck and V. Merken. *Tenside Deterg.* 17(1), 25-6 (1980). Cotton and polyester-cotton fabrics were laundered 20 times in hard water (360 ppm) at 85C in the presence of an anionic surfactant and 5g/l of a builder mixture containing sodium carbonate and various proportions of sequestering and dispersing agents. Additions of the dispersing agent sodium polyacrylate also reduce the insoluble salt build-up produced by sodium carbonate.

INFLUENCE OF PROMOTORS ON PHOTOCHEMICAL PROCESS OF SULPHOXIDATION OF N-PARAFFINS IN AQUEOUS SYSTEM. A. Kowalski et al. *Tenside Deterg.* 17(1), 27-9 (1980). The effect of promotors on the photochemical process of sulphoxidation of the fraction C_{14}-C_{17} of n-paraffins in the presence of water was studied. The best promotors in respect to the increase of reaction rate and the product composition were found to be: chloroform, pentachlorethane and acetic acid anhydride added at the rate of 2%v/v.

PRIMARY AND TOTAL BIODEGRADATION OF LINEAR ALKYLBENZENESULFONATES. C. Divo and G. Cardini. *Tenside Deterg.* 17(1), 30-6 (1980). The degradation of single linear alkylbenzenesulfonates was studied using a microorganism able to utilize synthetic surfactants as the sole carbon source at concentrations of about 1 gram per liter. The process was followed by parallel determination of the methylene blue active substance and of the total dissolved organic carbon. The microorganism attacks and rapidly degrades part of the alkyl chain, with the production of sulfophenyl-monocarboxylic acids, which were isolated and characterized.

Calendar

AOCS NATIONAL MEETINGS

Annual Meeting, 1981: May 17-21, Fairmont Hotel, New Orleans, LA.

Annual Meeting, 1982: May 2-6, Sheraton Centre, Toronto, Ontario, Canada.

Annual Meeting, 1983: May 8-12, Chicago Marriott, Chicago, IL.

AOCS SHORT COURSES

AOCS Short Course on Soaps and Detergents, Sept. 14-17, 1980, Hershey, PA.

1980

1980 Conference on International Cosmetic Regulations, Sept. 27, 1980, Venice, Italy, sponsored by the International Federation of Societies of Cosmetic Chemists.

Symposium: "Sensory Evaluation of Product Performance," Oct. 20-22, 1980, sponsored by the Society of Cosmetic Chemists, Hilton Hotel, Stratford-upon-Avon, England. Contact: M. Callingham, 56 Kingsway, London WC2B 6DX, England.

Eighth Residential Postgraduate Course in Cosmetic Science, Nov. 9-15, 1980, sponsored by the Society of Cosmetic Scientists, Palace Court Hotel, Bournemouth, England. Contact: M. Callingham, 56 Kingsway, London WC2B 6DX, England.

Society of Cosmetic Chemists Annual Scientific Meeting, Dec. 11-12, New York City. Contact: Program Cochairmen, Robert L. Goldemberg or Harvey S. Schnur, c/o Society of Cosmetic Chemists, 1995 Broadway, Suite 1701, New York, NY 10023.

1981

The Soap and Detergent Association Industry Convention, Jan. 28-Feb. 1, 1981, Boca Raton Hotel & Club, Boca Raton, FL.

"Color Technology for Management," June 16-17, 1980, sponsored by Rensselaer Color Measurement Laboratory, Rensselaer Polytechnic Institute, Troy, NY. Contact: Office of Continuing Studies, Rensselaer Polytechnic Institute, Troy, NY 12181.

"Advances in Color Technology," June 23-27, 1980, sponsored by Rensselaer Color Measurement Laboratory, Rensselaer Polytechnic Institute, Troy, NY Contact: Office of Continuing Studies, Rensselaer Polytechnic Institute, Troy, NY 12181.

Fourth International Conference on Surface and Colloid Science, July 5-10, 1981, Jerusalem, Israel. Contact: A.S. Kertes, Institute of Chemistry, The Hebrew University, Jerusalem, Israel.

Chemical Marketing Research Association national meeting, Sept. 20-23, 1981, Dunfey's, Hyannise, MA. Contact: CMRA, 139 Chestnut Ave., Staten Island, NY, 10305 (tele: 212 727-0550).

1982

The Soap and Detergent Association Industry Convention, Jan. 27-31, 1982, Boca Raton Hotel & Club, Boca Raton, FL.

1983

The Soap and Detergent Association Industry Convention, Jan. 26-30, 1983, Boca Raton Hotel & Club, Boca Raton, FL.

1984

The Soap and Detergent Association Industry Convention, Jan. 1984, Boca Raton Hotel & Club, Boca Raton, FL. □

(SD&C 118)

Abstracts

EDITOR: S. Koritala

ABSTRACTORS: J.C. Harris, M.G. Kokatnur, F.A. Kummerow, G. List, B. Matijasevic, K.D. Mukherjee, D.B.S. Min, R.A. Reiners and P.Y Vigneron

Biochemistry and nutrition

EXPERIMENTAL NEPHROTIC SYNDROME INDUCED IN THE RAT BY PUROMYCIN AMINONUCLEOSIDE: HEPATIC SYNTHESIS OF NEUTRAL LIPIDS AND PHOSPHOLIPIDS FROM ^{3}H-WATER AND ^{3}-H-PALMITATE. E. Gherardi and S. Calandra (Istituto di Patologia Generale, Universitá degli Studi di Modena Via Campi 287, 41100 Modena, Italy) *Lipids 15*(2), 108-12 (1980). Experimental nephrotic syndrome (ascites, proteinuria, hypoalbuminemia, and hyperlipidemia) was induced in male Wistar rats by seven daily subcutaneous injections of puromycin aminonucleoside (20 mg/kg). Hepatic lipogenesis from ^{3}H-water and ^{3}H-palmitate was investigated in nephrotic and pair fed control rats by using liver slices. The difference in hepatic lipogenesis between nephrotic and control rats was even more pronounced if the data were corrected for the total liver weight which was significantly increased in the nephrotic rats (11.3 $\pm$.3 vs. 8.5 $\pm$.1 g, p $<$.001). These findings indicate that the synthesis of neutral lipids from both ^{3}H-water and ^{3}H-palmitate is elevated in rat with aminonucleoside-induced nephrotic syndrome. The possible role of the increased hepatic lipogenesis in the pathogenesis of the nephrotic hyperlipidemia is discussed.

REGULATION OF CHOLESTEROL BIOSYNTHESIS IN CULTURED CELLS BY PROBABLE NATURAL PRECURSOR STEROLS. G.F. Gibbons, C.R. Pullinger, H.W. Chen, W.K. Cavenee and A.A. Kandutsch (Med. Res. Council, Lipid Metabolism Unit, Hammersmith Hosp., London, W12 OHS, United Kingdom) *J. Biol. Chem. 255*(2), 395-400 (1980). Lanosterol derivatives bearing an additional oxygen function at carbon 32 are generally considered to be natural cholesterol precursors. Two such oxysterols, 5α-lanost-8-ene-3β,32-diol and 3β-hydroxy-5α-lanost-8-en-32-al, strongly inhibited the incorporation of [1-^{14}C] acetate into sterols in cultures of Chinese hamster lung cells, mouse L-cells, and fetal mouse liver cells. In the latter two cell types, this inhibition could be accounted for predominantly by a decrease in the activity of the enzyme hydroxymethylglutaryl-CoA reductase (NADPH) (EC 1.1.1.34). In the lung cells, lanosterol 14α-demethylation was also suppressed. The oxygenated lanosterols had no effect on reductase activity in a mutant line of Chinese hamster lung cells selected for resistance to 25-hydroxycholesterol (cholest-5-ene-3β,25-diol). In these cases, only lanosterol 14α-demethylation was inhibited. All these effects were also observed with the Δ^7 bond isomers of the above compounds. Studies of the metabolism of the 32-oxygenated lanostenols in L-cells and mouse fetal liver cells revealed that, while all were converted into C_{27} sterols (including cholesterol), to a varying degree, each was more extensively metabolized to both polar and nonpolar products. Similar products also arose during the metabolism of each of the C-15 epimers of 5α-lanost-8-ene-3β, 15-diol and of their respective Δ^7 bond isomers.

INTERACTION OF HUMAN PLASMA HIGH DENSITY LIPOPROTEIN HDL_2 WITH SYNTHETIC SATURATED PHOSPHATIDYLCHOLINES. E.L. Gong and A.V. Nichols (Donner Lab., Lawrence Berkeley Lab., Univ. of Calif., Berkeley, CA 94720) *Lipids 15*(2), 86-90 (1980). The interaction of human plasma high density lipoprotein HDL_2 (d 1.063-1.125 g/ml) with sonicated dispersions of synthetic saturated phosphatidylcholines, dipalmitoyl- ($diC_{16}PC$), dimyristoyl- ($diC_{14}PC$), didodecanoyl- ($diC_{12}PC$), didecanoyl- ($diC_{10}PC$), and dioctanoyl- (diC_8PC) L-alpha phosphatidylcholine, was investigated. Incubation (4.5 hr, 37 C) of HDL_2 with $diC_{14}PC$, $diC_{12}PC$, $diC_{10}PC$ and diC_8PC followed by gradient gel electrophoresis or preparative ultracentrifugation resulted in a redistribution of apolipoprotein A-I (apoA-I). The extent of redistribution depended on the molar ratio of the phospholipid to HDL_2 in the incubation mixture. Redistributed apoA-I occurred as lipid-free apoA-I and/or as complexes of apoA-I with phosphatidylcholine. Increasing the length of time of ultracentrifugation of the interaction mixtures did not increase the extent of redistribution. No redistribution of apoA-I was detected following incubation and gradient gel electrophoresis or preparative ultracentrifugation of mixtures of HDL_2 with dispersions of $diC_{16}PC$.

BIOSYNTHESIS OF CHOLIC ACID IN RAT LIVER: FORMATION OF CHOLIC ACID FROM 3α, 7α, 12α-TRIHYDROXY- AND 3α, 7α, 12α, 24-TETRAHYDROXY-5β-CHOLESTANOIC ACIDS. J. Gustafsson (Dept. of Chem., Karolinska Institutet, Stockholm, Sweden) *Lipids 15*(2), 113-21 (1980). Conversion of 3α7α12α-trihydroxy-5β-cholestanoic acid into 3α,7α,12α24-tetrahydroxy5β-cholestanoic and cholic acids was catalyzed either by the mitochondrial fraction fortified with coenzyme A, ATP, $MgCl_2$ and NAD or by the combination of microsomal fraction and 100,000 x g supernatant fluid fortified with coenzyme A, ATP and NAD. 24-Hydroxylation and formation of cholic acid occurred at similar rates with the 25R- and the 25S-forms of 3α,7α,12α-trihydroxy-5β-cholestanoic acid. The 25R- and 25S-forms of 3α,7α,12α-trihydroxy- and 3α,7α,12α,24-tetrahydroxy-5β-cholestanoic acids were administered to bile fistula rats. Labeled cholic acid was isolated from the bile. The initial specific radioactivity of cholic acid was higher and the disappearance of radioactivity more rapid after administration of 3α,7α,12α-trihydroxy-5β-cholestanoic acid than of 3α,7α,12α,24-tetrahydroxy-5β-cholestanoic acid. The findings are discussed in relation to the assumed pathway for side chain cleavage in cholic acid biosynthesis.

THE EFFECTS OF DIETARY PHOSPHORUS, VITAMIN D_3, AND 25-HYDROXY VITAMIN D_3 LEVELS ON FEED INTAKE, PRODUCTIVE PERFORMANCE, AND EGG AND SHELL QUALITY IN TWO STRAINS OF FORCE-MOLTED WHITE LEGHORNS. R.M.G. Hamilton (Animal Res. Inst., Res. Branch, Agri. Canada, Central Experimental Farm, Ottawa, Canada) *Poult. Sci. 59*(3), 598-604 (1980). A total of 576 force-molted hens were used to investigate the effect of dietary phosphorus level, and the source and level of vitamin D_3 on egg production and shell quality. The experiment was a 2x2x2x3 factorial design with two strains of hens, two levels of available phosphorus (.34 and .60%), and two sources of D_3 (25-hydroxy vitamin D_3 and vitamin D_3) at three levels (8, 16, 24 μg/kg); the diets were provided *ad libitum* for 84 days (from 646 to 730 days of age). Egg and shell quality measurements were taken on eggs laid over a 4-day period when the hens were 727 days of age. Dietary phosphorus level, and D_3 source and level had no significant (P $>$.05) effect on 730-day body weight, feed intake and efficiency, egg production and yield, shell weight, percent shell, shell weight per unit surface area, Haugh units, blood spots, egg specific gravity, nondestructive deformation, compression fracture force, and shell thickness. There were significant differences among strains for the aforementioned variables except feed intake, egg weight, Haugh units, and blood spot incidence. Few interactions were found between the main effects. Results indicate that no improvement in shell quality of eggs from force-molted hens was obtained by decreasing the level of phosphorus or substituting 25-hydroxy vitamin D_3 for vitamin D_3 in the diet.

STEREOCHEMISTRY OF THE SIDE CHAIN OXIDATION OF 5β CHOLESTANE-3α,7α,12α-TRIOL IN MAN. R.F. Hanson, P. Szczepanik-VanLeeuwen, and G.C. Williams (Dept. of Internal Med., Univ. of Minn., Minneapolis, MN 55455) *J. Biol. Chem. 255*(4), 1483-5 (1980). Previous *in vitro* studies have shown that the oxidation of the side chain of bile acid precursors can start with either microsomal or mitochondrial enzyme systems. The microsomal system oxidizes the terminal methyl group (C-26) of the side chain that originates from C-2 of mevalonic acid, and the mitochondrial system oxidizes the terminal methyl group (C-27) derived from C'-3 of mevalonic acid. We administered [2-^{14}C] mevalonic acid to a patient with a complete bile fistula and isolated from the bile 3α,7α,12α-trihydroxy[1,7,15,22,26-^{14}C] 5β-cholestanoic acid, a precursor in the synthesis of cholic acid which has undergone partial oxidation of the side chain. This study suggests that the major pathway of side chain oxidation of 5β-cholestane-3α,7α,12α-triol starts with hydroxylation of the methyl group derived from C'-3 of mevalonate (C-27) by mitochondrial enzymes. It is also concluded that the 3α,7α,12α-trihydroxy-5β-cholestan-27-oic acid formed in man has the R configuration at carbon 25.

HYDROXYLATIONS IN BIOSYNTHESIS AND METABOLISM OF BILE ACIDS. CATALYTIC PROPERTIES OF DIFFERENT FORMS OF CYTOCHROME P-450. R. Hansson and D. Wikvall (Dept. of Pharmaceutical Biochem., Univ. of Uppsala, Biomed. Center, Box 578, S-751 23 Uppsala, Sweden) *J. Biol. Chem. 255*(4), 1643-9 (1980). Three fractions of cytochrome P-450 were prepared from liver microsomes of phenobarbital-treated rabbits. The fractions appeared homogeneous on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and contained 11 to 19 nmol of cytochrome P-450/mg of protein. Two of the fractions were apparently identical with cytochromes P-450 LM_2 and LM_4. Two fractions of cytochrome P-450 were purified from liver microsomes of phenobarbital-treated rats. The fractions were apparently homogeneous on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and contained 14 to 18 nmol of cytochrome P-450/mg of protein. Two fractions of cytochrome P-450 were purified from liver microsomes of cholestyramine-treated rats. The fractions showed one major band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and contained 14 to 15 nmol of cytochrome P-450/mg of protein.

FATTY ACID SYNTHETASES FROM *EUGLENA GRACILIS*. SEPARATION OF COMPONENT ACTIVITIES OF THE ACP-DEPENDENT FATTY ACID SYNTHETASE AND PARTIAL PURIFICATION OF THE β-KETOACYL-ACP SYNTHETASE. R.W. Hendren and K. Bloch (James Bryant Conant Laboratories, Harvard Univ., Cambridge, Mass. 02138) *J. Biol. Chem. 255*(4), 1504-8 (1980). The component enzymes of the chloroplast-associated, acyl carrier protein (ACP)-dependent, fatty acid synthetase (FAS-II) from *Euglena gracilis* have been independently examined by gel filtration chromatography of crude extracts from photoautotrophic cells. Under certain conditions, several of the *E. gracilis* FAS-II component activities may aggregate noncovalently to form a weak complex. A partial purification of the β-ketoacyl-ACP synthetase by ammonium sulfate fractionation, DEAE-cellulose chromatography, and hydroxylapatite chromatography resulted in its complete separation from the enoyl-ACP reductase activity. When the separated β-ketoacyl-ACP synthetase and enoyl-ACP reductase activities were recombined and subjected to gel filtration chromatography, the two activities migrated distinctly and with lower apparent molecular weights, 118,000 and 56,500, respectively, than when similarly measured in the crude extract.

LIVER AND SERUM LIPIDS AND LIPOPROTEINS OF RATS FED 5% L-LYSINE. P. Hevia and W.J. Visek (Schools of Basic Med. Sci. and Clin. Med., Univ. of Illinois, Urbana, IL 61801) *Lipids 15*(2), 95-9 (1980). Soybean protein and casein supplemented with 1% Arginine were compared for their ability to prevent fatty livers caused by excess dietary Lysine. The concentrations of serum lipids and lipoproteins of rats fed 5% Lysine and having fatty livers were also compared with those of rats fed the identical diet but lacking fatty livers when killed. Overall, excessive dietary Lysine caused fatty livers which were prevented by varying the diet or length of feeding. Excess Lysine feeding altered lipoprotein metabolism shown by decreased serum HDL and a substantial elevation in LDL. The latter was more apparent when the fat accumulation in liver was less severe or absent. The data suggest that the fatty liver from Lysine excess is probably unrelated to increased fat mobilization from storage, decreased fat oxidation or to a major block in the transport of triglycerides from the liver to the circulation.

DECREASED FORMATION OF PROSTAGLANDINS DERIVED FROM ARACHIDONIC ACID BY DIETARY LINOLENATE IN RATS. D.H. Hwang and A.E. Carroll (Human Nutr. and Food, School of Home Economics, Louisiana State Univ., Baton Rouge, LA 70803) *Am. J. Clin. Nutr. 33*(3), 590-7 (1980). Accumulated evidence now suggests that availability of precursor acid is an important factor controlling the biosynthesis of prostaglandins (PG's). Since linolenic acid inhibits the conversion of linoleic acid to arachidonic acid (PGE_2, $PGF_2\alpha$, and thromboxane A_2 precursor), rats receiving more linolenic acid are expected to have less arachidonic acid and thus less PG's synthesized from arachidonic acid than those receiving linoleic acid alone. Essential fatty acid-deficient rats, induced by feeding hydrogenated coconut oil diet for 15 weeks, were divided into six groups and fed graded amounts of purified methyl linolenate for 6 weeks. Each group of rats showed that the level of arachidonic acid in serum lipids and serum concentrations of PG's synthesized from arachidonic acid by platelets decreased as the amount of dietary linolenate increased. This indicated that biosynthesis of PG's in platelets can be influenced by the availability of precursors, and thus it can be modified by the manipulation of dietary fatty acids.

MECHANISM OF ACTION OF MILK LIPOPROTEIN LIPASE AT SUBSTRATE INTERFACES: EFFECTS OF APOLIPOPROTEINS. R.L. Jackson, F. Pattus and G. de Haas (Dept. of Pharmacology and Cell Biophysics, Biochem. and Med., Univ. of Cincinnati Coll. of Med., Cincinnati, OH 45267) *Biochemistry 19*(2), 373-8 (1980). The mechanism of action of bovine milk lipoprotein lipase was studied by using a monomolecular film of 1,2-didecanoylglycerol. The apparent rate of hydrolysis of diglyceride increased with increasing surface pressures above 12 mN/m; the enzyme was inactive at pressures less than 12 mN/m. We have measured the effects of four plasma apolipoporteins (apoC-II, apoC-III, apoA-I, and apoE), bovine serum albumin, porcine pancreatic colipase, heparin, and NaCl on the kinetics of lipid hydrolysis. At a surface pressure of 15 mN/m, all of the proteins, with the exception of colipase, gave increased enzyme activity compared to lipase alone; apoC-II gave maximal activation. At 25 mN/m, apoC-II at concentrations of less than 0.25 μ g/mL showed a specific activation, whereas the other proteins had no effect. Heparin activated at both high and low surface pressures; NaCl had little or no effect in this system. At a higher concentration of apoC-II (0.50 μ g/mL), the apoprotein inhibited the enzyme. The addition of apoC-III, apoA-I, or apoE (final concentration 0.25 μ g/mL), but not albumin or colipase, to apoC-II (0.25 μ g/mL) caused an increase in surface pressure of 5-6 mN/m and an apparent rate which was less than half that found for lipase alone, suggesting that all of the apoproteins inhibit the apoC-II specific activation.

EFFECT OF SUCROSE POLYESTER ON FECAL STEROID EXCRETION BY 24 NORMAL MEN. R.J. Jandacek, F.H. Mattson, S. McNeely, L. Gallon, R. Yunker and C.J. Glueck (Miami Valley Lab., Procter and Gamble Co., Cincinnati, OH 45247) *Am. J. Clin. Nutr. 33*(2), 251-9 (1980). The effect of the nonabsorbable fat-like substance, sucrose polyester (SPE), on neutral steroid excretion was determined in 24 healthy men. Initially the subjects received for 10 days a basal diet that was high (800 mg/day) or low (300 mg/day) in cholesterol or for 21 days a basal diet containing $<$50 mg cholesterol per day. These diets were isocaloric. Over three subsequent, consecutive periods of 10 days each, 8, 16, or 25 g/day of liquid SPE or 19, 38, or 62 g/day of an 80/20 mixture of SPE and completely hydrogenated palm oil was added to the diet. The amounts of C27 steroids, i.e., cholesterol and its conversion products formed by intestinal bacteria, were measured in the feces of each subject. In each individual, the intestinal bacteria converted β-sitosterol to its characteristic products in the same proportion as was cholesterol. The addition of SPE to the diet caused a lesser amount of the sterols to be modified by the bacteria. This could be measured more readily in the converter population. There, each gram of SPE that was ingested resulted in 1.1% decrease in conversion for those who received liquid SPE and a 0.6% decrease for those who received the SPE-hydrogenated palm oil mix. The decrease in conversion is a probable consequence of the sterols being dissolved in the oil phase of SPE in the lumen of the intestinal tract and hence unavailable to the bacteria.

PROTEIN-LIPID INTERACTIONS. STUDIES OF THE M13 COAT PROTEIN IN DIMYRISTOYLPHOSPHATIDYLCHOLINE VESICLES USING PARINARIC ACID. D. Kimelman, E.S. Tecoma, P.K. Wolber, B.S. Hudson, W.T. Wickner and R.D. Simoni (Dept. of Biological Sciences and the Dept. of Chem., Stanford Univ., Stanford, CA 94305) *Biochemistry 18*(26), 5874-80 (1979). Addition of the M13 virus coat protein to dimyristoylphosphatidylcholine vesicles decreases the amplitude of the lipid bilayer phase transition observed with parinaric acid fluorescence intensity. According to these measurements, the solid-phase bilayer is disordered by the protein, while the fluid phase is not appreciably affected. The results are consistent with a perturbation of the bilayer by the coat protein at low temperature. The structure of the bilayer near the protein results in a spatial distribution of *trans*-parinaric acid such that this quencher avoids the donor protein. The intensity and partitioning/quenching behavior used to characterize the effect of the protein on the solid bilayer do not reveal any bilayer structural changes for the fluid-phase bilayer. However, fluorescence polarization measurements with parinaric acid reveal a marked decrease in the rotational mobility of the probe relative to that of the fluid bilayer. The utility of parinaric acid fluorescence methods in the study of membrane-bound proteins is emphasized.

THE EFFECT OF ISOMERIC *TRANS*- 18:1 ACIDS ON THE DESATURATION OF PALMITIC, LINOLEIC AND EICOSA-8,11,14-TRIENOIC ACIDS BY RAT LIVER MICROSOMES. M.M. Mahfouz, S. Johnson and R.T. Holman (The Hormel Inst., Univ. of Minn., Austin, MN 55912) *Lipids 15*(2), 100-7 (1980). The inhibitory effects of the positional isomers of *trans*- 18:1 acids on the desaturation of palmitic acid to palmitoleic (Δ9-desaturase), linoleic to γ-linolenic(Δ6-desaturase) and eicosa-8,11,14-trienoic to arachidonic acid (Δ5-desaturase) were investigated. These *trans*-18:1 acids were found to be inhibitory for the microsomal Δ6-, Δ9- and Δ5-desaturases of rat liver. The position of the double bond in the *trans*-18:1 acids seems to be important in determining the degree of inhibition. At inhibitor/substrate ratio of 3:1, the Δ6-desaturase was most strongly inhibited by *trans*-Δ3, -Δ4, -Δ7 and -Δ15-18:1 isomers, whereas the Δ9-desaturase was most strongly inhibited by

trans-Δ3, -Δ5, -Δ7, -Δ10, -Δ12, -Δ13 and -Δ16 isomers. At inhibitor/substrate ratio of 6:1, the Δ5-desaturase was most strongly inhibited by Δ3-, Δ9-, Δ13- and Δ15-isomers. When 18:0 was added to the incubations of 16:0, 18:2 and 20:3 at the same I/S ratios used for the *trans*-18:1 acids, weak inhibition for Δ9-desaturase and no inhibition for Δ5- and Δ6-desaturases was observed.

IMMUNOCHEMISTRY OF HUMAN VERY LOW DENSITY LIPOPROTEINS: APOLIPOPROTEIN C-III. S.J.T. Mao, P.K. Bhatnagar, A.M. Gotto, Jr., and J.T. Sparrow (Dept. of Med., Baylor College of Med. and The Methodist Hosp., Houston, TX 77030) *Biochemistry 19*(2), 315-20 (1980). Apolipoprotein C-III (apoC-III) is a major protein constituent of human plasma very low density lipoproteins (VLDL) and a minor constituent of high density lipoproteins (HDL). The apoprotein is a singly polypeptide chain of 79 amino acids and occurs in several forms differing only in their content of sialic acid. In the present report a quantitative radioimmunoassay (RIA) has been developed in order to study the immunochemical properties of apoC-III. Two individual rabbit antisera were used. Since the conformation of the apoprotein has drastically changed upon the addition of dimyristoylphosphatidylcholine (DMPC), this finding indicates that the gross conformational change of apoC-III_1 does not affect the immunochemical properties and that the antigenic reactive sites are probably located at the surface in apoC-III_1-DMPC complexes. The immunoreactivity of apoC-III was also found to be approximately the same in HDL or VLDL as that of the delipidated apoHDL or apoVLDL. Thus, the antigenic sites of apoC-III must be fully exposed on the surface of the lipoproteins.

STUDIES ON THE POLYMORPHISM OF HUMAN APOLIPOPROTEIN A-I. A.C. Nestruck, G. Suzue and Y.L. Marcel (Institut de Recherches Cliniques de Montréal, 110, Avenue des Pins ouest, Montréal H2W 1R7, Québec, Canada) *Biochim. Biophys. Acta 617*(1), 110-21 (1980). Upon preparative isoelectric focussing of human apo-HDL, four major forms of apolipoprotein A-I have been isolated. These forms of apolipoprotein A-I were shown to have identical migration on polyacrylamide gel electrophoresis, molecular weights of 26 000 on sodium dodecyl sulfate gel electrophoresis and a common antigenicity with antisera against apolipoprotein A-I or A-I_1. Each form had very similar amino acid compositions with the exception of form apolipoprotein A-I_4. All forms but apolipoprotein A-I_4 were activators of lecithin:cholesterol acyltransferase, the latter was inhibitory to the reaction. From these results, it was concluded that apolipoprotein A-I_1, A-I_2 and A-I_3 are equivalent forms of apolipoprotein A-I whereas apolipoprotein A-I_4 is different or heterogeneous. Upon refocussing, the polymorphs were shown to be stable at their p*I* and not affected by changes in concentration and by the presence of urea or ampholytes. Exposure of a form of apolipoprotein A-I to alkaline pH partially regenerated the original heterogeneity; however, apolipoprotein A-I_4 regenerated from apolipoprotein A-I_1 did not contain isoleucine, which further demonstrates form apolipoprotein A-I_4 heterogeneity.

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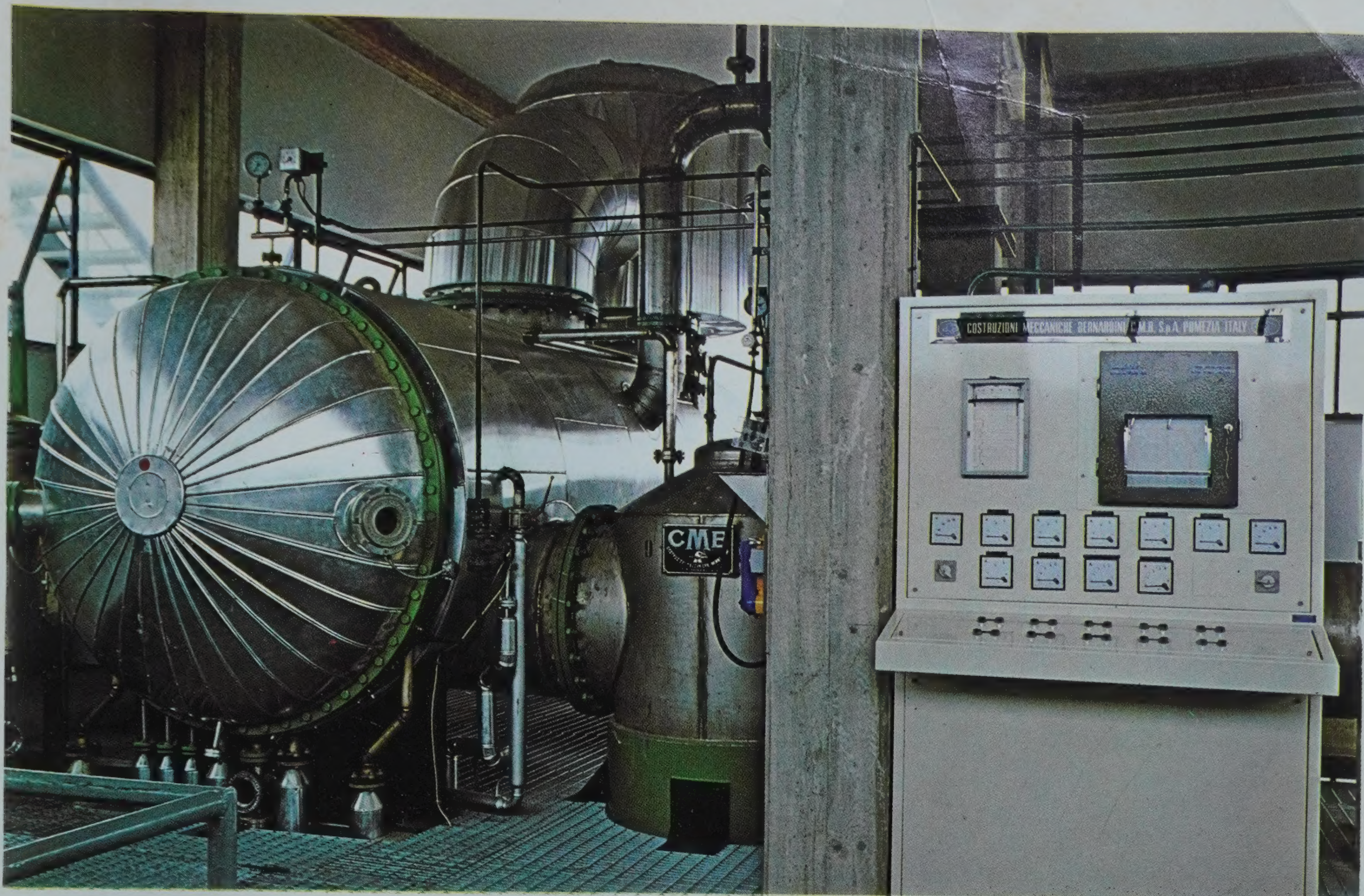
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